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ԲԱՆԲԵՐ ՍՏՈՄԱՏՈԼՈԳԻԱՅԻ ԵՎ ԴԻՄԱԾՆՈՏԱՅԻՆ ՎԻՐԱԲՈՒԺՈՒԹՅԱՆ
Գիտագործնական հանդես

Գլխավոր խմբագիր - Հակոբյան Գ.Վ.
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RESEARCH ARTICLE

A STUDY TO EVALUATE THE BONE AUGMENTATION WITH LEUKOCYTE AND PLATELET RICH FIBRIN PREPARATION IN EXTRACTION PLACEMENTS AND ITS INFLUENCE ON PERI IMPLANT TISSUE

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ABSTRACT

Purpose: A study was done to evaluate bone augmentation and its influence on peri-implant tissue in implant-supported prosthesis in extraction and immediate placement scenarios.

Material and methods: A total of 20 patients underwent atraumatic extraction with immediate endosteal implant placement and immediate provisional loading. The patients were categorized as Group I: 10 patients indicated for atraumatic extraction and immediate endosteal implant placement and immediate provisional loading and Group II: 10 patients were indicated for atraumatic extraction with socket augmentation and immediate provisional loading. Volumetric changes of the sites were recorded at 3 months and 6 months post-operative with a D-SLR camera NIKON D 3100 with a Macro lens (1:1).

Results: Statistical analysis showed a significant increase in the papilla in the augmented site than non-augmented site. Soft tissue healing however was similar.

Conclusion: Within the limitations of the study, it can be safely concluded that soft tissue healing is better with provisionalisation even without socket grafting in the anterior esthetic zone. Nevertheless, there are long-term benefits of socket grafting.

Keywords: Immediate provisionalisation, Peri-implant esthetics, Socket augmentation

1. INTRODUCTION

Implant treatment has evolved a lot over the years. Two-stage and one-stage implant placement are very common among the population and they are not as case sensitive as the immediate implants. Even though Immediate implants clearly reduce patient visits and interventions, there are various factors influencing the success of the treatment. Hard and soft tissue architecture, implant stability, three-dimensional positioning, socket augmentation, and provisionalisation, to name a few.[1] Immediate implant in extraction sockets with immediate provisionalisation has its own challenges. The “aesthetic zone,” that is the anterior teeth has high visibility and patients are highly sensitive to the appearance. The papilla plays a major role which in turn is determined by the interproximal architecture of bone of the adjacent teeth, the facial mucosa contour, the root proximity the phenotype of the soft tissue, the reason for the loss of teeth, buccal bone fenestrations.[2] In addition to saving time, the reason for still opting for immediate placement and provisionalisation is to potentially maximize the preservation of hard and soft

tissues. A well-done provisional restoration for immediate extraction cases can not only regain theconfidence of patients but also provide a blueprint for the tissues to restore themselves to their original midfacial, gingival, and interdental papillary architecture.[3]

Tarnow et al., 30 years back stated that the distance from the contact point of the teeth to the crestal bone level should be five mm or less to ensure complete interdental papillary fill.[4] When there is loss of hard tissue around the peri-implant marginal bone area, per implant mucosa can also be affected or compromised by a break in seal and thus, affect the health.[5] This could inevitably cause aesthetic failures. It is a known fact that the level of interdental papilla relies also on the bone next to the adjacent teeth. In cases with buccal bone fenestration or dehiscence, immediate extraction warrants bone augmentation.[6] To date no study has compared papilla volume of immediately placed implants and loading without bone augmentation and immediate placement of implants with loading along with bone augmentation. The study aimed to compare the volume of interproximal papilla and thereby the zenith of immediate loading of implants in fresh extraction sites before and after bone augmentation on the day of loading and at the time interval of 3 months and 6 months.

2. MATERIAL AND METHODS

Twenty patients with unrestorable teeth were selected from the outpatients at the Department of Prosthodontics and Crown and Bridge at AB Shetty Memorial Institute of Dental Sciences, Mangalore India. Patients were deemed unsuitable for this study if they presented with one or more of the following: poor oral hygiene, smoking more than 10 cigarettes per day, uncontrolled diabetes, immune diseases, and head and neck radiation in the preceding 12 months. Localized dehiscence or fenestration, and signs of acute periodontal infection at the surgical site were also excluded from the study. The study was approved by the Institutional Ethics Committee at AB Shetty Memorial Institute of Dental Sciences (ABSM/EC/306/2023).

2.1 Presurgical preparation

Informed consent was taken after explaining the benefits and the risks associated with the procedure. Post-operative care also was strictly enforced. A papillary bleeding index (Muhlemann H.R. 1977)[8] was recorded to record the health of the gingival tissues. A preoperative X-ray was taken to evaluate the bone architecture, thereafter selection of the implants was done. Diagnostic models and wax mock-ups were done for provisionalisation.

Liquid Platelet Concentrate Preparation: 10cc of blood was collected to obtain the liquid platelet concentrate preparation Leukocyte and Platelet Rich Fibrin(L-PRF). The centrifuge (Duo Quattro) was equipped with preprogrammed operational panel for the centrifugation of blood. Thus,

L-PRF (2300 rpm for 12mins) was obtained as per manufacturers recommendations.This was then used within 30 minutes of preparation along with the alloplastic graft for the augmentation group.

2.2 Surgical phase

Patients were prescribed prophylactic antibiotic therapy (Amoxicillin 500 mg) The implant surgery was performed by a single operator. All implants were categorized into two groups. The most common implants were Sandblasted Acid-etched implants. Grade 2 and Grade 5 Ti was used for the study. Ankylos implants (Dentsply, Mannheim, Germany); and Straumann implants with SLA surface (Straumann, Basal, Switzerland), including bone level or tissue level implants were the predominant implant systems used.

Group I (Control Group N=10) included 10 patients who received no bone augmentation after the immediate placement.

Group II (intervention group n=10) included 10 patients who received bone augmentation with alloplastic material (sybograf-plus) with a composition of 70% nanocrystalline hydroxyapatite and 30% beta-tricalcium phosphate and LPRF.

In a sterile operatory, the implant surgery began with the disinfection of the facial skin with Povidone – (Iodine Solution IP (Betadine) paint). Induction of local anaesthesia was carried out using Lignospan special (2% lidocaine with 1:80,000 Adrenaline). The procedure commenced with the atraumatic extraction of the teeth requiring replacement with the help of sharp periotomes. Osteotomy was done on the thick palatal wall of the extraction socket for reasons such as prevention of labial perforation along with bypassing buccal resorptive patterns of the maxilla.[7] Immediate implant placement was performed. Surgical sites were prepared to achieve stability of at least 35 Ncm which was a prerequisite for immediate loading and provisionalisation. As per the implant abutment torque standards the abutment was torqued, 15 Ncm in this case Group II: the standard protocol mentioned above for osteotomy was maintained. Subsequently following implant placement, bone graft (alloplast+LPRF) was condensed in the implant-socket gap (>2mm jumping distance).

The abutment was torqued and the provisionals of the tissue surface is well contoured into the material.

2.3 Prosthetic phase

Provisional restoration was done using light cure resin-based composite material (Protemp 4, 3M ESPE). The provisional was kept out of occlusion for the transitional phase of three months. A metal-ceramic implant-supported crown was fabricated and fixed onto the implant with the final torque established by the manufacturers (25Ncm) at 3 months post-operative. Photographs were taken with D-SLR camera Nikon D 3100 and macro lens along with a set magnification of 1:1 by the same operator. Soft tissue profile photographs were taken pre and post-implant placement, provisional loading phase, and also during 3 months and 6 months post crown fixation.

The papillary fill was measured by drawing an imaginary line joining the most apical part or the zeniths of the adjacent teeth. A perpendicular line was then drawn to connect the contact point in the software Adobe Photoshop version 10. The line was equally divided into parts. The papillary volume fill was measured using the papilla index (JEMT 1997)[9] (figure 1). The papillary fill was measured by joining the zeniths of the adjacent teeth and then drawing a line perpendicular to it till the contact point in the software Adobe Photoshop version 8. The data obtained i.e. the papilla level changes on the mesial and distal side of the implant was tabulated in Microsoft Excel Sheets and the statistical analysis was performed using SPSS 15 software.

2.4 Statistical analysis

Statistical analysis was performed using IBM SPSS version 23. Comparisons of gingival fill at various time intervals were done using the Friedman test.

3. RESULTS

3.1 Comparison of gingival fill between the control group and Augmented Group

The Mean of the control and augmented group at baseline on the mesial side was 10.50. Mean of the distal side of the control and the augmented group was 10.50. The mean of 3 months distal and mesial of control was 11.30 mean of 3 months distal and mesial of augmented group was 9.70. The mean of the mesial side at 6 months of the control group was 11.65 and the augmented side was 9.35. and the mean of the distal side of the control group was 11.75 and the augmented side was 9.25. There was no statistically significant difference in the gingival fill between the control group and the bone graft group on both mesial and distal sides.

Table 1. Comparison of gingival fill between the control group and Augmented Group

Ranks						
	Group	**N**	**Mean Rank**	**Sum of Ranks**	**Mann-Whitney**	**P value**
Mesial_ Baseline	Control	10	10.50	105.00	50.000	1.000
	AUGMENTED GROUP	10	10.50	105.00		
	Total	20				
Distal_ Baseline	Control	10	10.50	105.00	50.000	1.000
	AUGMENTED GROUP	10	10.50	105.00		
	Total	20				
Mesial_3 months	Control	10	11.30	113.00	42.000	.473
	AUGMENTED GROUP	10	9.70	97.00		
	Total	20				
Distal_3 months	Control	10	11.30	113.00	42.000	.473
	AUGMENTED GROUP	10	9.70	97.00		
	Total	20				
Mesial_6 months	Control	10	11.65	116.50	38.500	.350
	AUGMENTED GROUP	10	9.35	93.50		
	Total	20				
Distal_6 months	Control	10	11.75	117.50	37.500	.304
	AUGMENTED GROUP	10	9.25	92.50		
	Total	20				

Table 2. Comparison of gingival fill in the control group at different time intervals -Friedman Test

Side	Sample1–Sample2	Test statistic	Std error	Std test statistic	significance	Adjusted significance/p-value
Mesial_control	Baseline–3months	**-9.700**	**4.851**	**-2.117**	**0.034**	**0.205**
	Baseline–6months	**-18.700**	**4.851**	**-4.082**	**0.000**	**0.000**
	3months–6months	**-9.000**	**4.851**	**-1.965**	**0.049**	**0.297**
Distal_control	Baseline–3months	-10.800	4.640	-2.328	0.020	0.120
	Baseline–6months	-22.900	4.640	-4.936	0.000	0.000
	3months–6months	-12.100	4.640	-2.608	0.009	0.055
Mesial_augmented	Sample1–Sample2	**Test statistic**	**Std error**	**Std test statistic**	**significance**	**Adjusted significance/p-value**
	Basleine-3months	-3.850	5.015	-0.768	0.443	1.00
	Baseline -6months	-8.800	5.015	-1.755	0.079	0.476
Distal_augmented	Sample 1–Sample2	**Test statistic**	**Std error**	**Std test statistic**	**significance**	**Adjusted significance/p-value**
	Baseline–3months	-4.200	5.027	-0.836	0.443	1.00
	Baseline–6months	-10.250	5.027	-2.039	0.041	0.249

Control group: The mean at different intervals at the mesial side in the control group was 1.30, 2.40, and 3.40 respectively at baseline 3 months and 6 months. There was a statistically significant difference in gingival fill on the mesial side of the control group. The difference in gingival fill between baseline and 6 months was statistically significant. In the control group, On the distal side, the mean was 1.25, 2.35, and 3.55 at baseline, 3 months, and 6 months respectively. There was a statistically significant difference in gingival fill on the distal side of the control group. The difference in gingival fill between baseline and 6 months was statistically significant.

Augmented group: The mean of the mesial side of the augmented group was 1.55, 2.05, and 2.70 at baseline, 3 months, and 6 months. There was a statistically significant difference in gingival fill on the mesial side of the augmented group. The difference in gingival fill was not statistically significant. The means of the distal side were 1.60, 2.05, and 2.70 at baseline, 3 months, and 6 months respectively. The difference in gingival fill between not statistically significant.

4. DISCUSSION

The maxillary anterior region is by far one of the hardest regions to rehabilitate. Not just the obvious reason of aesthetics and speech but also the bony architecture is way too compromised and unpredictable. The most common cause of tooth loss in the anterior region is trauma and tooth decay. So immediate extraction and placement could be a viable choice. Chen et al in their study has emphasized the hard tissue and soft benefits of extraction and immediate placement such as faster healing time.[10] Lang et al. in their systematic review concluded that that extraction and immediate placement have successful and predictable outcomes. Initial loss of bone in the first should be overcompensated with sub crestal placement.[11] Although promising aesthetic and functional results are documented in extraction placement by many authors, soft tissue healing is always compromised after an extraction placement, due to lack of periodontal recovery and inevitable bone loss.[12,13] To compensate grafting the jumping distance and provisionalising on the same day can also form a foundation for good soft tissue architecture.[14] The present study was intended to evaluate the volume of the papillary fill and gingival zenith and compare it with non-augmented sites.

The gingival was periodically evaluated at three months and six months follow-up. The present study evaluated the effect of bone augmentation on the soft peri-implant tissues. The bone graft used in the study is an alloplastic material (sybograf-plus) with a composition of 70% nanocrystalline hydroxyapatite and 30% beta tricalcium phosphate along with autologous blood formulation LPRF i.e. Leukocyte and platelet-rich fibrin. The presence of hydroxyapatite crystals and beta tricalcium phosphate with sizes varying from 300-500 microns helps in faster bone formation and also acts as a scaffold for the generation of new bone thus providing mechanical stability to the overlying soft

tissues.[14-20] LPRF has known to have faster wound healing properties. Thus, a composite graft should ideally provide faster healing.[20-23]

Even though there are benefits of augmentation, these procedures can be tedious and financially challenging, thus for jumping distances of less than 2mm we can still just provide provisionals that would manipulate the papilla and also in the grafted site mechanically stabilise the graft. Making sure the provisional is out of occlusion a constant follow-up is necessary to check on the intaglio surface of the provisional and modifications can be done as and when required. A screw-retained prosthesis would be an ideal option.[24–26] In this study there was a mean increase in the volume of interdental papilla and complete papillary fill in both control (group I) and augmented group (group II) at 3 months and 6-month follow-up. Nevertheless, the mean increase in the augmented site was more both in the distal as well as the mesial side comparable to the result of various studies.[10,14,25-27] In a study done by Rojas et al.,[28] he concluded that the success rate is slightly greater in the case of immediately placed implants. Immediate placement and loading with a provisional in the anterior region of the maxilla may be considered a promising treatment outcome based on the present short-term study of 6 months in function with a 95% survival rate.

The study had no long term follow up of the soft tissue profile. The index used also does not give any elaboration on the nature, consistency and the area-wise volume of the periodontium. Since the interpretation of photographs are very subjective, there is a reason to believe more sensitive tests are required to fulfil the accuracy.

5. CONCLUSION

Within the limitation of the study, the following conclusion has been drawn: LPRF and alloplastic grafting material can be considered a viable option for developing bone in extraction sockets. Provisionalisation added to the bone growth and soft tissue contouring. A well-placed implant and socket grafting can deliver esthetic results in the anterior zone.

DECLARATIONS

Ethical approval and consent to participate – The study was approved by the Institutional Ethics Committee at AB Shetty Memorial Institute of Dental Sciences (ABSM/EC/306/2023) and consent was taken from all participants before data collection.

Availability of data and material

All data generated or analyzed during this study are included in the published article.

Competing interest

The authors declare that there are no competing interest.

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None

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ORIGINAL RESEARCH

ASSESSMENT OF EFFICACY OF UV LIGHT BASED NOVEL AIR SANITIZING DEVICE IN THE REDUCTION OF ORAL MICROFLORA DURING ORTHODONTIC PROCEDURES

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ABSTRACT

Purpose: Due to the possibility of airborne contamination by biological diseases, implementing severe bio-safety standards inside dental clinic settings has become a primary requirement. Given the potential biological risk of cross-contamination from saliva, blood, aerosol, and/or droplets created during dental treatments, evaluating the qualitative and quantitative reduction of oral microbiota in the presence of UV radiation is critical. This study aims to assess the efficacy of UV tunnel in a qualitative and quantitative reduction in oral microflora during aerosol (debonding) and non-aerosol (bonding) generating orthodontic procedures in a dental clinical setup.

Materials and Methods: The microbial load in the dental clinic's air was measured using the settling plate technique. Petri dishes were standardized and placed throughout the room at various heights and distances. The bacterial/viral load was determined qualitatively and quantitatively using petri dish samples.

Results: Total Viable Count (TVC) was assessed at different time intervals, and except for 24 and 48 hours, it was statistically significant. Total Mold Count (TMC) was assessed at different levels from the patient's oral cavity, and a significant difference was seen between the patient level, the doctor, and the assistant level, even after 48 hours. However, there was no statistical difference at the doctor and patient level in debonding and bonding generating groups.

Conclusion: UV tunnel devices show a statistically significant decline amongst the viable molds but also refrain from chances of recontamination.

1. INTRODUCTION

Dentists are among the most exposed health care professionals, being exposed to a wide variety of aerosols generated during dental procedures. Reduced microbial contamination is an important feature of infection control strategies. The rate of hospital-acquired infections has increased dramatically in the past few years owing to multi-drug resistant strains of certain micro-organisms[1,2].

Harrel and Molinari et al. reported aerosols are composed of a variety of components viz, saliva, nasopharyngeal secretions, blood, dental plaque, tooth scrapping, water from dental unit water pipelines, and other dental materials used during the procedure. Aerosols almost reach a distance of 1.5 m from the patient's oral cavity, at the breathing level of the operating dentist, adjacent surfaces, facial masks, and

face shields[3]. Aerosols and splatter are two common ways for contaminated particles to spread during dental operations. They differ primarily in droplet size, diffusion capacity in the air, and ability to survive in external environmental conditions prior to reaching a host. Aerosols are less than 50 µm in diameter and freely suspended in the air for extended periods; smaller size and lightweight aid them to easily penetrate lungs, further presenting a potential risk for respiratory infections[4,5].

Sterilization with UV radiation is an excellent method of bio-decontamination. Recent research has shown a significant reduction in qualitative and quantitative assessments of oral microbiota when UV radiation is used. The effect of UV irradiation varies depending on characteristics such as organic load, nature of the oral pathogen, intensity, dose, distance from the device, exposure period, direct line of sight from the device or shaded exposure, lamp placement, room size and shape, and surface to be irradiated. Newer touchless sterilization approaches provide an incremental benefit over manual practices by limiting cross-transmission of oral pathogens via environmental surfaces, though literature evidence of the invention of certain pathogens still remains limited[6,7].

Hence, the present pilot study was envisaged with a view to assessing the efficacy of UV tunnel in a qualitative and quantitative reduction in oral microflora during aerosol (debonding) and non-aerosol (bonding) generating orthodontic procedures in a dental clinical setup.

2. MATERIAL AND METHODS

This pilot study was conducted to assess the feasibility of an in-vitro study, which will be conducted in the future. The sample size was calculated with 10% of the original sample and with a total of 16 samples.

Selection criteria:

Inclusion criteria for orthodontic procedures –

1. Aerosol generating procedures – Debonding procedures
2. Non-aerosol generating procedures – Bonding procedures

Exclusion criteria for orthodontic procedures –

1. Patients with systemic illness or conditions
2. Patients with acute or chronic respiratory symptoms or conditions
3. Patients with a history of illness in the last 30 days
4. Any other aerosol-generating or non-aerosol-generating orthodontic procedure

Air sampling

The microbial load in the air of the dental clinic was measured using the settled plate technique. The 90 mm diameter Petri dishes containing either blood agar (Colombia blood agar base (Hach, Loveland, USA), supplemented with 5% sheep blood) or R2A agar (Hach) were exposed to the air for 30 minutes at 80 cm height from the floor. The air was then sampled at six moments during a normal day of patient treatment: 1) before the first procedure. The room was unoccupied for at least 24 hours; 2) 15 minutes during the dental procedure; and 3) 30 minutes after the final treatment (the room was unoccupied during that time). 4) 1 hour after the final procedure (the room was unoccupied during that time). 5) 6 hours after the final procedure (the room was unoccupied during that time). 6) 24 hours after the final procedure (the room was unoccupied during that time). 7) 48 hours after the final procedure (the room was unoccupied during that time). The plates were placed in four locations: 1) on the patient's chest, at doctor's levels; 2) at the level of the nostrils of the treating dentist; 3) at the level of the nostrils of the chairside assistant; 4) at the level of the nostrils of the attendant.

UV Tunnel

The UV tunnel device was 8 8-watt Philips/Osram tube having a fan air volume of 2940 LPM and a fan life of about 50,000 hours. The fan had high speed and high volume with a wavelength of 253.6 nm. The tube life is about 9000 hours, and the AC voltage is about 240V AC. The UV tunnel was kept in the operatory room and the bacterial/viral load was assessed qualitatively and quantitatively using the petri dishes samples.

Methodology

A single-chair operatory was chosen in a dental clinic setup, and x number of sites were selected. While choosing the sites, it would be ensured that there is sufficient and proper distribution of plates at various locations both vertically as well as horizontally, i.e., some Petri dishes were spread around the room at different heights and varying distances and were standardized.

Step 1: There was assessment of the room prior to sanitization. This was carried out to assess the resting baseline of the room.

Step 2: Complete sanitization of the room was carried out with either fumigation, UV lights, or certain disinfectant sprays to ensure that the viral/bacterial load of the room becomes as close to zero. Since, zero was the baseline of each room.

Step 3: Conduction of various aerosol and non-aerosol generating procedures in the sanitized operatory.

Step 4: Collection of the sample from various sites at varying time intervals within the operatory room.

Step 5: Culturing the sample with qualitative and quantitative analysis.

The Process Of Group Allocation

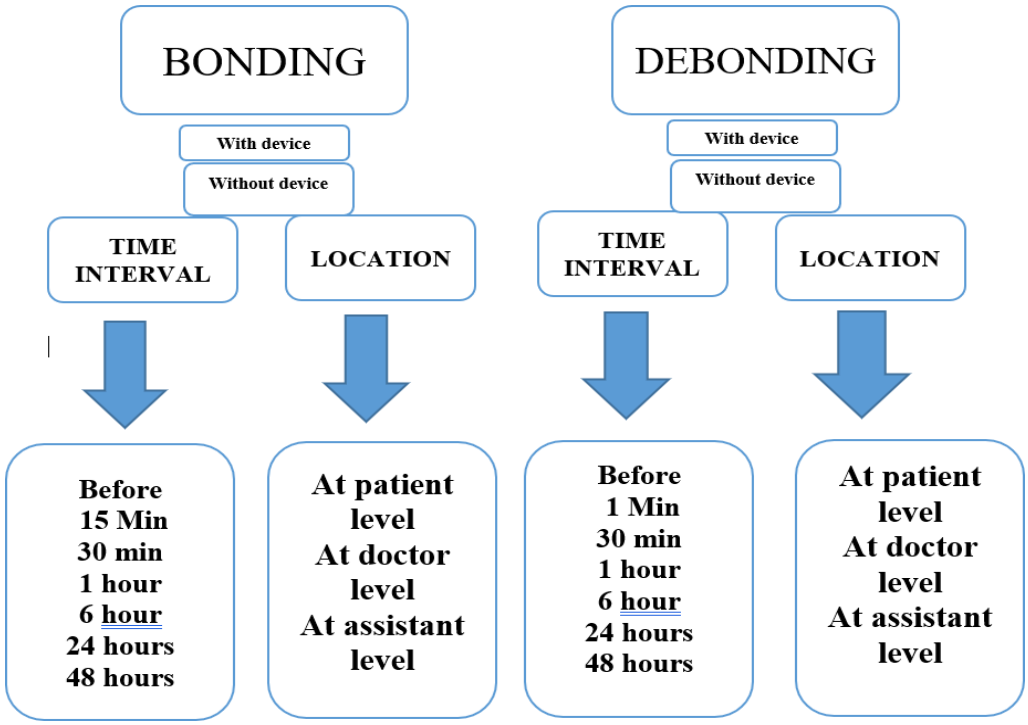


Figure 1. Pain intensity in Group NSAIDs and Group TCA in follow up

The collected samples were sent for culturing and the culture growth was examined at the above-mentioned intervals to assess the change in the quality and quantity of microflora at various time intervals. The cultures were assessed until the bacterial/viral load of the petri-dishes reaches to baseline zero again. When the cultures consistently arrive at zero baseline for 2 or 3 consecutive days, then it would be assumed that the viral/bacterial load are no longer multiplying, i.e, they have reached their stable position and this was end the 1st phase of the experiment. The procedure was repeated before and after usage of UV tunnel for determining its efficacy in both bonding as well as debonding and the results were analyzed both qualitatively as well as quantitatively.

STATISTICAL ANALYSIS

Data were analysed using descriptive statistics. The acquired data was analyzed using SPSS version 21 software. The current study included both descriptive and inferential statistical analysis. Continuous measurement findings were provided as Mean ± SD, whereas categorical measurements were presented as a percentage. The level of significance was set at p = 0.05, and any value less than or equal to 0.05 was considered statistically significant. Chi square analysis was performed to determine the relevance of study parameters on a categorical scale.

TABLE 1. Mean Scores for TVC & TMC at different time intervals

	Group	N	Mean	Std. Deviation
Efficacy 15 min	TVC	8	12.2375	10.42012
	TMC	8	34.0650	22.23930
Efficacy 30 min	TVC	8	27.6413	13.37588
	TMC	8	53.4988	17.14093
Efficacy 1 hours	TVC	8	48.5688	8.32253
	TMC	8	63.1663	16.53527
Efficacy 6 hours	TVC	8	67.1050	1.36582
	TMC	8	70.6075	13.10542
Efficacy 24 hours	TVC	8	81.2988	3.10276
	TMC	8	76.9263	12.09200
Efficacy 48 hours	TVC	8	86.8188	4.31143
	TMC	8	90.9513	8.30007

The data was collected at different time intervals, from 15 minutes to 48 hours. **Table 1** shows the mean scores for both groups at these different time intervals. At every interval, TVC showed lower values than TMC except at 24 hours, 81.29 ± 3.1 & 76.92 ± 12.09, respectively.

TABLE 2. Comparative Evaluation Of TVC And TMC At Different Time Intervals

Dependent Variable	Group	Group	Mean Difference	Sig.
TVC efficacy	15 min	30 min	-15.40375*	.005
		1 hour	-36.33125*	.000
		6 hours	-54.86750*	.000
		24 hours	-69.06125*	.000
		48 hours	-74.58125*	.000
	30 min	15 min	15.40375*	.005
		1 hour	-20.92750*	.000
		6 hours	-39.46375*	.000
		24 hours	-53.65750*	.000
		48 hours	-59.17750*	.000
	1 hour	15 min	36.33125*	.000
		30 min	20.92750*	.000
		6 hours	-18.53625*	.000
		24 hours	-32.73000*	.000
		48 hours	-38.25000*	.000
	6 hours	15 min	54.86750*	.000
		30 min	39.46375*	.000
		1 hour	18.53625*	.000
		24 hours	-14.19375*	.012
		48 hours	-19.71375*	.000
	24 hours	15 min	69.06125*	.000
		30 min	53.65750*	.000
		1 hour	32.73000*	.000
		6 hours	14.19375*	.012
		48 hours	-5.52000	.741
	48 hours	15 min	74.58125*	.000
		30 min	59.17750*	.000
		1 hour	38.25000*	.000
		24 hours	19.71375*	.000
		48 hours	5.52000	.741

TMC efficacy	15 min	30 min	-19.43375	.147
		1 hour	-29.10125*	.007
		6 hours	-36.54250*	.000
		24 hours	-42.86125*	.000
		48 hours	-56.88625*	.000
	30 min	15 min	19.43375	.147
		1 hour	-9.66750	.813
		6 hours	-17.10875	.258
		24 hours	-23.42750*	.046
		48 hours	-37.45250*	.000
	1 hour	15 min	29.10125*	.007
		30 min	9.66750	.813
		6 hours	-7.44125	.929
		24 hours	-13.76000	.495
		48 hours	-27.78500*	.011
	6 hours	15 min	36.54250*	.000
		30 min	17.10875	.258
		1 hour	7.44125	.929
		24 hours	-6.31875	.963
		48 hours	-20.34375	.115
	24 hours	15 min	42.86125*	.000
		30 min	23.42750*	.046
		1 hour	13.76000	.495
		6 hours	6.31875	.963
		48 hours	-14.02500	.474
	48 hours	15 min	56.88625*	.000
		30 min	37.45250*	.000
		1 hour	27.78500*	.011
		24 hours	20.34375	.115
		48 hours	14.02500	.474

Table 2 shows the comparative evaluation of the two groups at different time intervals.

TABLE 3. Descriptive statistics for the TVC group at different time intervals with and without UV device

Time intervals	Group	N	With UV Device		Without UV Device	
			Mean	Std. Deviation	Mean	Std. Deviation
TVC Before	Bonding	4	175.7500	12.01041	173.7500	1.01841
	Debonding	4	204.7500	8.13941	207.7500	9.13041
TVC 15 min	Bonding	4	143.2500	23.47161	140.1500	11.47161
	Debonding	4	192.5000	5.56776	187.2000	5.59076
TVC 30 min	Bonding	4	106.2500	10.01249	116.2500	9.01249
	Debonding	4	172.2500	5.90903	198.2500	5.87003
TVC 1 hour	Bonding	4	80.5000	11.67619	81.5000	10.67619
	Debonding	4	116.0000	5.35413	126.0000	5.35413
TVC 6 hours	Bonding	4	57.7500	2.62996	87.7600	2.72996
	Debonding	4	67.2500	2.21736	77.4500	3.21736
TVC 24 hours	Bonding	4	28.2500	2.50000	35.2500	9.50000
	Debonding	4	43.5000	3.00000	78.5000	3.56000
TVC 48 hours	Bonding	4	17.7500	5.85235	65.7500	9.89235
	Debonding	4	33.0000	4.54606	90.0000	8.49606

Table 3 shows the mean scores of the TVC group in Bonding and Debonding before the intervention and at all the time intervals. The values for debonding are higher than the bonding values, and all with UV device values progressively decrease from before intervention 175.75 ± 12 bonding, 204.75 ± 8.1 debonding to 48 hours 17.75 ± 5.8 & 33 ± 4.5, respectively. Such a trend was not observed without the UV device values. However, higher values were observed in debonding than in bonding.

TABLE 4. Comparative evaluation of TVC at different distances with & without UV device

Parameter	Group	With UV device			Without UV device		
		T	MD	P value	T	MD	P value
TVC Before	Bonding	.812	-29.00000	.007*	-.078	-32.00000	.007*
	Debonding						
TVC 15 min	Bonding	4.286	-49.25000	.006*	-.126	-37.25000	.006*
	Debonding						
TVC 30 min	Bonding	.536	-66.00000	.000*	-.196	-58.00000	.000*
	Debonding						
TVC 1 hour	Bonding	11.022	-35.50000	.001*	-.063	-43.50000	.001*
	Debonding						
TVC 6 hours	Bonding	.020	-9.50000	.001*	-.078	-43.50000	.001*
	Debonding						
TVC 24 hours	Bonding	.794	-15.25000	.000*	-.126	-35.25000	.000*
	Debonding						
TVC 48 hours	Bonding	.332	-15.25000	.006*	-.196	-15.25000	.006*
	Debonding						

*Statistically significant ($p < 0.05$)

Table 4 shows the mean difference between bonding and debonding scores at different time intervals. The difference found was significant statistically in both the with & without UV groups at every time interval.

TABLE 5. Descriptive statistics for the TVC group at different time intervals with debonding & bonding procedure

Time interval	Group	N	Debonding		Bonding	
			Mean	Std. Deviation	Mean	Std. Deviation
TVC Before	With device	4	171.2500	12.01041	165.7500	1.01841
	Without device	4	206.2500	8.13961	201.7500	9.13041
TVC 15 min	With device	4	146.1500	13.47161	130.1500	31.47161
	Without device	4	196.5000	9.56776	197.2000	5.59076
TVC 30 min	With device	4	111.2500	60.01249	166.2500	6.01249
	Without device	4	162.2500	05.90903	178.2500	9.87003
TVC 1 hour	With device	4	78.5000	12.67619	80.5000	11.67619
	Without device	4	106.0000	5.95413	136.0000	7.35413
TVC 6 hours	With device	4	77.7500	12.62996	87.9600	9.72996
	Without device	4	65.2500	21.21736	77.8500	6.21736
TVC 24 hours	With device	4	45.2500	12.50000	35.2500	5.50000
	Without device	4	43.5000	3.00000	98.5000	6.56000
TVC 48 hours	With device	4	27.7500	5.85235	65.7500	4.89235
	Without device	4	36.0000	1.54606	80.0000	9.49606

Table 5 depicts the Mean & SD for debonding and bonding devices at different time intervals. For the debonding device gradual decrease was seen in the scores as the time increased, whereas no such observation was made in the bonding group. However, in both groups scores for without the UV device were higher than in with device.

Table 6. Comparative evaluation of TVC at different distances with debonding & bonding procedures.

Parameter	Group	Debonding			Bonding		
		T	MD	P value	T	MD	P value
TVC Before	With device	.812	-9.00000	.000*	-.078	-22.00000	.009*
	Without device						
TVC 15 min	With device	4.286	-37.25000	.006*	-.126	-47.25000	.008*
	Without device						
TVC 30 min	With device	.536	-45.00000	.010*	-.196	-98.00000	.000*
	Without device						
TVC 1 hour	With device	11.022	-65.50000	.001*	-.063	-33.50000	.001*
	Without device						
TVC 6 hours	With device	.020	-19.50000	.001*	-.078	-63.50000	.001*
	Without device						
TVC 24 hours	With device	.794	-25.25000	.010*	-.126	-75.25000	.000*
	Without device						
TVC 48 hours	With device	.332	-15.25000	.006*	-.196	-25.25000	.007*
	Without device						

Table 6 gives the mean difference in the scores for with and without UV devices at different distances with debonding & bonding procedures. The difference was statistically significant for all time intervals,

Table 7. Descriptive statistics for the TMC group at different time intervals with & without device

Parameter	Group	N	With device		Without device	
			Mean	Std. Deviation	Mean	Std. Deviation
TMC Before	**Bonding**	4	7.0000	.81650	8.0000	.81050
	Debonding	4	11.5000	4.35890	10.5000	7.35890
TMC 15 min	**Bonding**	4	5.2500	.50000	6.2500	1.50000
	Debonding	4	6.0000	3.55903	7.0000	5.55903
TMC 30 min	**Bonding**	4	3.7500	.95743	5.7500	2.95743
	Debonding	4	4.5000	3.10913	7.5000	1.10913
TMC 1 hour	**Bonding**	4	3.0000	.81650	3.0000	0.81650
	Debonding	4	3.2500	2.50000	3.2500	6.50000
TMC 6 hours	**Bonding**	4	2.2500	.50000	3.2500	.50000
	Debonding	4	2.7500	2.21736	3.7500	.21736
TMC 24 hours	**Bonding**	4	1.7500	.50000	2.7500	7.50000
	Debonding	4	2.0000	1.41421	2.0000	2.41421
TMC 48 hours	**Bonding**	4	.5000	.57735	.5000	3.57735
	Debonding	4	1.0000	.81650		

A similar analysis was done for the TMC group. The following tables show the results obtained.
For the TMC group, mean values at different time intervals in with UV device and without UV device group are given in **Table 7.**

Table 8. Comparative evaluation of TMC at different distances with & without device

Parameter	Group	With device			Without device		
		t value	MD	P value	t value	MD	P value
TMC Before	**Bonding**	-2.029	-4.50000	.089	-6.029	-4.50000	.081
	Debonding						
TMC 15 min	**Bonding**	-.417	-.75000	.691	-.717	-.75000	.871
	Debonding						
TMC 30 min	**Bonding**	-.461	-.75000	.661	-.962	-.75000	.461
	Debonding						
TMC 1 hour	**Bonding**	-.190	-.25000	.855	-.990	-.25000	.805
	Debonding						
TMC 6 hours	**Bonding**	-.440	-.50000	.675	-.840	-.50000	.615
	Debonding						
TMC 24 hours	**Bonding**	-.333	-.25000	.750	-.343	-.25000	.150
	Debonding						
TMC 48 hours	**Bonding**	-1.000	-.50000	.356	-1.000	-.50000	.206
	Debonding						

The mean difference at each time interval in TMC group for with UV device and without UV device groups are given in **Table 8.**

Table 9. Descriptive statistics for the TMC group at different time intervals with debonding & bonding procedures.

Parameter	Group	N	Debonding		Bonding	
			Mean	Std. Deviation	Mean	Std. Deviation
TMC Before	With device	4	6.0000	1.00650	9.0000	.78050
	Without device	4	10.5000	2.35890	9.5000	4.35890
TMC 15 min	With device	4	6.2500	.57000	6.2500	1.50000
	Without device	4	6.0000	3.55903	6.0000	3.55903
TMC 30 min	With device	4	4.7500	.85743	5.7500	2.95743
	Without device	4	4.5000	2.10913	6.5000	0.10913
TMC 1 hour	With device	4	3.0000	.71650	4.0000	0.81650
	Without device	4	2.2500	2.70000	3.2500	1.50000
TMC 6 hours	With device	4	3.2500	.50800	4.2500	.57000
	Without device	4	3.7500	2.71736	3.7500	.21736
TMC 24 hours	With device	4	2.7500	.50000	1.7500	7.57000
	Without device	4	3.0000	1.41021	2.0000	1.41021
TMC 48 hours	With device	4	1.5000	.67735	.5000	3.57135
	Without device	4	1.5000	.87650	1.0000	2.81650

Table 9 shows the mean and SD values at different time intervals with debonding and bonding procedures. The values decrease progressively as the time interval increases.

4.DISCUSSION

Aerosols are likely to be produced during proximal stripping, banding, debonding, and other procedures in the active phase of orthodontic therapy. Upon completion of the active orthodontic treatment period, the bands and bonded attachments are extracted. The excess adhesive on the teeth can be eliminated using specialized pliers. Utilize a dome-tapered tungsten carbide bur in a high-speed dental handpiece, operating at about 30,000 rpm, to eliminate any residual adhesive from the tooth surface. To avert damage or necrosis of the dental pulp, a water spray is affixed to cool the handpiece tip with water. This subsequently results in the development of aerosols around the operatory, raising infection concerns for the orthodontist, dental assistants, and the patient[8–10]. Mitigating airborne infections is essential in dental environments since they can disseminate bacteria and viruses, presenting a health hazard to orthodontists, patients, and staff. HEPA air filters are exceptionally efficient at diminishing bioaerosols, hence mitigating airborne illnesses. Recent advancements, such as UV air sanitizers devoid of a HEPA filter (UV Tunnel device), assert that the likelihood of re-contamination is diminished as they emit inactive or killed microorganisms alongside purified air, thereby serving as a catalyst for immune response among healthcare professionals, without the risk of disease causation or progression. Ten The efficacy was evaluated as one of the parameters at various time intervals for both TVC and TMC. The estimated mean ratings revealed that efficacy improved with the passage of time.

According to Day CJ (2008), the aerosols generated during orthodontic bonding and debonding are often inhaled irrespective of handpiece speed in the presence or absence of water coolant, and as a result, aerosol-generating orthodontic procedures should be briskly assessed for timely prevention of respiratory diseases and maintenance of systemic health of the orthodontists[13–15]. Efficacy was one of the factors measured at various time intervals for both TVC and TMC. According to the estimated mean scores, efficacy increased with time. Similarly, TVC and TMC were examined at various time intervals, with the exception of 24 and 48 hours, showing a significant difference in both groups. The mean TVC count decreased as time passed. The bonding group showed the greatest reduction when compared to the non-bonding group with devices. The TMC was assessed at different levels from the patient's oral cavity. There was a significant difference seen between patient level and doctor as well as assistant level even after 48 hours. However, there was no statistical difference at the doctor and patient level in both debonding and bonding generating groups.

Bonding and debonding techniques should be the main emphasis of realistic management in practice, and it is essential to carefully choose procedures and apply

safety measures based on each patient's unique demands[16]. Taken together, particulate matter production, toxicity, and microbiologic issues should all be taken into account when implementing widespread and uniform occupational controls to limit aerosol creation in orthodontic practices[17,18].

5. CONCLUSION

An array of aerosols continues to circulate in moderately turbulent air within the dental setup; therefore, it is largely required to reduce bio-burden during orthodontic treatments in order to protect orthodontists from hazardous aerosol exposure. Furthermore, UV radiation, due to its natural ability to damage DNA by producing dimerization of pyrimidines, is extremely effective in lowering the bio-burden of contaminated aerosol. As a result of this evidence, it is very obvious that UV tunnel devices not only demonstrate a statistically significant drop among viable mould, but also reduce the likelihood of re-contamination.

DECLARATIONS

Ethical approval and consent to participate

Not Applicable

Availability of data and material

All data generated or analyzed during this study are included in the published article.

Competing interest

The authors declare that there are no competing interests.

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RESEARCH ARTICLE

EVALUATING ANTIMICROBIAL EFFICACY OF DIFFERENT TREATMENT MODALITIES ON DENTAL IMPLANT SURFACE: AN IN VITRO STUDY

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ABSTRACT

Background: Dental implants for replacing missing teeth has become standard practice in dentistry. Biological complications like peri-implantitis is common and involves biofilm-induced inflammation and bone loss around implants.

Material and Methods: Sixty standardized titanium discs (10 mm diameter, 2 mm thickness) were fabricated from grade 4 commercially pure titanium. All discs underwent sandblasting and acid etching to create a surface topography representative of contemporary dental implants. Surface characterization was performed using scanning electron microscopy (SEM) to ensure standardization across specimens. The discs were sterilized by gamma irradiation prior to biofilm formation.

Results: Er:YAG laser and photodynamic therapy achieved the highest bacterial reductions (94.3% and 92.1%).However, Er:YAG laser treatment also caused noticeable surface alterations, including melting and re-solidification patterns and smoothing of some surface irregularities.

Conclusion: Photodynamic therapy caused minimal surface alteration compared to other modalities. It may offer the best balance between antimicrobial efficacy and surface preservation in peri-implantitis management.

Keywords: Dental implants; peri-implantitis; biofilm; decontamination; photodynamic therapy; Er:YAG laser; chlorhexidine; air polishing

1. INTRODUCTION

The use of dental implants for replacing missing teeth has become standard practice in contemporary dentistry, with high long-term survival rates. However, the increased prevalence of implant-supported restorations has been accompanied by a rise in biological complications such as peri-implant mucositis and peri-implantitis. Peri-implantitis is an inflammatory condition affecting the tissues surrounding dental implants, characterized by inflammation of the peri-implant mucosa and progressive loss of supporting bone[1–4]. This pathological condition affects approximately 20% of patients with dental implants, posing significant challenges to long-term implant success and patient oral health[5].

The primary etiological factor for peri-implant diseases is bacterial biofilm accumulation on implant surfaces. The microbial composition associated with peri-implantitis resembles that of chronic periodontitis, with predominance of gram-negative anaerobic bacteria including *Porphyromonas gingivalis, Tannerella forsythia, Fusobacterium nucleatum, and Prevotella intermedia.* Unlike natural teeth, dental implants lack a periodontal ligament and cementum, resulting in different host responses to bacterial challenge. Additionally, the micro-roughened surfaces of modern implants, while beneficial for osseointegration, create an environment conducive to bacterial adhesion and biofilm formation[6,7].

Effective management of peri-implantitis necessitates thorough decontamination of implant surfaces to eliminate pathogenic microorganisms and create conditions favorable for re-osseointegration or soft tissue reattachment[2]. The complex topography of dental implants, characterized by threads, microgrooves, and varying surface treatments, presents significant challenges for conventional mechanical debridement alone[6,8]. Consequently, numerous adjunctive decontamination methods have been developed and investigated over the past decades.

Chemical decontamination approaches using antimicrobial agents such as chlorhexidine have been widely studied. Chlorhexidine (CHX), a cationic bisbiguanide, demonstrates broad-spectrum antimicrobial activity against both gram-positive and gram-negative bacteria through disruption of bacterial cell membranes[1]. Its substantivity allows a prolonged antimicrobial effect after application. However, concerns regarding cytotoxicity to osteoblasts and potential alterations to implant surface properties have been raised[5].

Laser therapy has emerged as a promising decontamination approach, with various types of lasers investigated for peri-implantitis treatment. The Er:YAG laser, in particular, has shown efficacy in biofilm removal without causing significant thermal damage to surrounding tissues when used with appropriate parameters[9]. Its ability to access areas difficult to reach through mechanical means makes it an attractive option for implant surface decontamination[10–12].

Photodynamic therapy (PDT) combines a photosensitizing agent (typically a dye such as methylene blue) with light of a specific wavelength to generate reactive oxygen species that exert antimicrobial effects. Several studies have reported significant bacterial reduction on implant surfaces following PDT application without adverse effects on surface properties[12]. The non-invasive nature of PDT and the absence of bacterial resistance development are notable advantages of this approach.

Air polishing systems utilize pressurized air, water, and abrasive powders to mechanically remove biofilm from implant surfaces. Recently, low-abrasive powders such as glycine have been introduced to minimize damage to implant surfaces while maintaining cleaning efficacy. Studies have demonstrated that air polishing with glycine powder can effectively reduce bacterial load on various implant surfaces with minimal surface alterations.

Other emerging approaches include ozone therapy, which utilizes the strong oxidizing properties of ozone to eliminate microorganisms;[6] hydrogen peroxide photolysis, which generates hydroxyl radicals with potent antimicrobial activity;[13] and plasma of argon treatment, which has shown promise in implant surface decontamination while preserving favorable surface characteristics for cell adhesion[14].

Therefore, this study aimed to comparatively evaluate the antimicrobial efficacy of four commonly used decontamination methods like chlorhexidine irrigation, Er:YAG laser treatment, photodynamic therapy, and air polishing with glycine powder on biofilm-contaminated titanium implant surfaces. The null hypothesis was that there would be no significant differences in bacterial reduction among the tested decontamination methods. Additionally, we sought to assess the effects of these treatments on implant surface topography, as surface alterations may influence subsequent cellular responses and healing potential.

2. MATERIAL AND METHODS

This in vitro experimental study was designed to evaluate and compare the antimicrobial efficacy of different treatment modalities on biofilm-contaminated titanium implant surfaces.

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Sixty standardized titanium discs (10 mm diameter, 2 mm thickness) were fabricated from grade 4 commercially pure titanium. All discs underwent sandblasting and acid etching to create a surface topography representative of contemporary dental implants. Surface characterization was performed using scanning electron microscopy (SEM) to ensure standardization across specimens. The discs were sterilized by gamma irradiation before biofilm formation.

Biofilm Formation

A three-species biofilm model consisting of *Porphyromonas gingivalis* ATCC 33277, *Fusobacterium nucleatum* ATCC 25586, and *Streptococcus mitis* ATCC 49456 was developed to simulate peri-implantitis biofilm. This combination was selected based on their established role in peri-implant diseases. Bacterial strains were cultivated in appropriate growth media under anaerobic conditions (80% N_2, 10% CO_2, 10% H_2) at 37°C for 48 hours. Bacterial suspensions were adjusted to 1×10^8 CFU/mL using spectrophotometric analysis.

Sterile titanium discs were placed in 24-well plates and incubated with 2 mL of the bacterial suspension for 72 hours at 37°C under anaerobic conditions to allow biofilm formation. Culture media were replaced every 24 hours. After incubation, discs were gently rinsed twice with phosphate-buffered saline (PBS) to remove non-adherent bacteria while preserving the biofilm structure.

Experimental Groups

The biofilm-contaminated titanium discs were randomly allocated to five experimental groups (n=12 per group):

1. Group CHX: Irrigation with 0.2% chlorhexidine digluconate solution for 60 seconds
2. Group ER: Er:YAG laser irradiation (2940 nm, 100 mJ/pulse, 10 Hz, 60 seconds, non-contact mode, distance 0.5 mm)
3. Group PDT: Photodynamic therapy using methylene blue (0.01%) as a photosensitizer for 60 seconds, followed by irradiation with a diode laser (660 nm, 100 mW/cm², 60 seconds)
4. Group AIR: Air polishing with glycine powder (mean particle size 25 μm, pressure 2 bar, distance 5 mm, 10 seconds)
5. Group CON: Irrigation with sterile saline solution for 60 seconds (control)

All treatments were performed by a single calibrated operator to minimize technique variability. After treatment, all specimens were rinsed with sterile PBS to remove residual agents or debris.

Microbiological Analysis

After decontamination procedures, six randomly selected specimens from each group were transferred to sterile tubes containing 10 mL of reduced transport fluid. Biofilms were dislodged by vortexing for 30 seconds, followed by ultrasonic dispersion for 60 seconds. Serial tenfold dilutions were prepared and plated on appropriate selective media. After anaerobic incubation for 7 days, colony-forming units (CFU) were counted by a blinded examiner. Bacterial reduction percentages were calculated relative to the control group.

Surface Topography Analysis

The remaining six specimens from each group were fixed in 2.5% glutaraldehyde, dehydrated in a graded ethanol series, and sputter-coated with gold for SEM analysis. Surface characteristics, including roughness, presence of debris, and surface alterations, were evaluated at various magnifications (500×, 1000×, and 3000×) by a trained examiner blinded to the treatment groups.

Statistical Analysis

Sample size calculation determined that 12 specimens per group would provide 80% power to detect a difference of 15% in bacterial reduction with $\alpha=0.05$. Statistical analysis was performed using SPSS software version 26.0. The Shapiro-Wilk test was used to assess data normality. Differences in bacterial reduction among groups were analyzed using one-way ANOVA followed by Tukey's post-hoc test. A p-value <0.05 was considered statistically significant.

3. RESULTS

The quantitative microbiological analysis revealed significant differences in bacterial reduction among the five experimental groups ($p<0.001$). **Table 1** presents the mean bacterial counts (CFU/mL) and bacterial reduction percentages for each treatment modality compared to the control group.

Table 1. Mean bacterial counts and bacterial reduction percentages across treatment groups

Treatment Group	Mean Bacterial Count (CFU/mL)	Log_{10} Reduction	Bacterial Reduction (%)
Control (Saline)	$2.7 \times 10^8 \pm 3.4 \times 10^7$	-	-
Chlorhexidine 0.2%	$4.6 \times 10^7 \pm 1.8 \times 10^7$	0.77	82.7
Er:YAG Laser	$1.5 \times 10^7 \pm 0.9 \times 10^7$	1.26	94.3
Photodynamic Therapy	$2.1 \times 10^7 \pm 1.3 \times 10^7$	1.11	92.1
Air Polishing	$3.1 \times 10^7 \pm 1.5 \times 10^7$	0.94	88.5

All decontamination methods demonstrated significantly higher bacterial reduction compared to the saline control ($p<0.001$). The Er:YAG laser treatment showed the highest bacterial reduction (94.3%), followed by photodynamic therapy (92.1%), air polishing (88.5%), and chlorhexidine irrigation (82.7%). Post-hoc analysis revealed that Er:YAG laser and photodynamic therapy groups exhibited significantly higher bacterial reduction compared to chlorhexidine ($p=0.023$ and $p=0.036$, respectively). No statistically significant difference was observed between Er:YAG laser and photodynamic therapy groups ($p=0.627$).

Analysis of individual bacterial species revealed varying susceptibilities to the different decontamination methods, as shown in **Table 2**.

Table 2. Bacterial reduction percentages by species across treatment groups

Treatment Group	*P. gingivalis* Reduction (%)	*F. nucleatum* Reduction (%)	*S. mitis* Reduction (%)
Chlorhexidine 0.2%	86.4 ± 5.2	84.1 ± 4.9	77.5 ± 6.3
Er:YAG Laser	95.8 ± 2.1	93.7 ± 3.5	93.4 ± 3.8
Photodynamic Therapy	94.3 ± 3.4	92.5 ± 4.2	89.6 ± 5.1
Air Polishing	90.2 ± 4.6	87.9 ± 5.3	87.5 ± 5.7
Control (Saline)	25.3 ± 7.2	32.7 ± 8.1	38.4 ± 7.9

P. gingivalis showed the highest susceptibility to all decontamination methods, particularly to Er:YAG laser treatment (95.8% reduction). *S. mitis* demonstrated relatively greater resistance to chlorhexidine (77.5% reduction) compared to other treatment modalities. The differences in bacterial reduction among species were statistically significant for chlorhexidine ($p=0.018$) but not for Er:YAG laser ($p=0.072$), photodynamic therapy ($p=0.086$), or air polishing ($p=0.111$).

SEM analysis revealed varying degrees of surface alterations across the experimental groups. The untreated control specimens showed dense biofilm covering the titanium surface, with characteristic bacterial morphologies visible. Chlorhexidine-treated specimens demonstrated significant biofilm reduction with minimal alterations to the underlying surface topography. Some residual bacteria and organic debris were observed in surface irregularities. Photodynamic therapy resulted in substantial biofilm elimination while preserving the original surface characteristics of the titanium discs.

Er:YAG laser treatment achieved the most substantial biofilm removal but caused noticeable surface alterations, including melting and resolidification patterns and smoothing of some surface irregularities. Air polishing with glycine powder effectively removed biofilm with moderate surface changes, primarily characterized by rounding of sharp edges and slight ablation of surface peaks.

Qualitative assessment of surface alterations on a scale of 0 (no alterations) to 3 (severe alterations) yielded mean scores of 0.5 ± 0.3 for chlorhexidine, 1.9 ± 0.5 for Er:YAG laser, 0.4 ± 0.2 for photodynamic therapy, and 1.2 ± 0.4 for air polishing.

4. DISCUSSION

The superior antimicrobial efficacy of Er:YAG laser observed in our study (94.3% bacterial reduction) aligns with findings from previous investigations. Giannelli et al. reported that Er:YAG laser effectively eliminated bacteria from titanium surfaces with minimal thermal damage when appropriate parameters were used[9]. Similarly, Schwarz F et al. demonstrated that Er:YAG laser irradiation at low energy settings achieved significant biofilm removal without causing detrimental thermal effects on implant surfaces[5]. The high efficacy of Er:YAG laser can be attributed to its specific wavelength (2940 nm), which is strongly absorbed by water and hydroxyapatite, resulting in photoacoustic and photomechanical effects that disrupt biofilm without substantial heat generation. However, our SEM analysis revealed noticeable surface alterations following Er:YAG laser treatment, suggesting that while antimicrobial efficacy is excellent, potential changes to surface properties must be considered.

Photodynamic therapy demonstrated comparable antimicrobial efficacy (92.1% bacterial reduction) to Er:YAG laser while causing minimal surface alterations. These findings corroborate those of Hayek et al., who reported that PDT using methylene blue and a low-power laser effectively reduced bacteria on implant surfaces without affecting surface characteristics[2,15]. The mechanism of PDT involves the generation of reactive oxygen species, particularly singlet oxygen, which causes oxidative damage to bacterial cell walls, membranes, and DNA. Importantly, PDT does not induce bacterial resistance, unlike many conventional antimicrobial agents. Our SEM analysis confirmed that PDT preserved the original surface topography of titanium specimens, suggesting it may offer an optimal balance between antimicrobial efficacy and surface preservation.

Air polishing with glycine powder demonstrated good antimicrobial efficacy (88.5% bacterial reduction) with moderate surface alterations. These results are consistent with those reported by Schwarz et al., who found that air polishing with glycine powder effectively removed biofilm from various implant surfaces with minimal abrasive effects[16]. The less aggressive nature of glycine particles compared to conventional air polishing powders (e.g., sodium bicarbonate) contributes to the preservation of surface integrity while maintaining cleaning efficacy. Nevertheless, our SEM observations of some surface modifications suggest caution when using this technique, particularly on highly textured implant surfaces.

Chlorhexidine irrigation, while significantly reducing bacterial load (82.7%), showed the lowest antimicrobial efficacy among the tested modalities. This finding aligns with several previous studies that have questioned the effectiveness of chlorhexidine as a standalone treatment for implant surface decontamination. Kotsakis et al. reported that chlorhexidine irrigation achieved only partial biofilm removal, particularly in areas with complex surface topography[16]. Additionally, concerns have been raised regarding the potential cytotoxic effects of chlorhexidine on osteoblasts and its impact on re-osseointegration. Kotsakis et al. demonstrated that chlorhexidine, even at low concentrations, exhibited cytotoxicity to osteoblasts and inhibited cell migration[16–20]. These findings suggest that while chlorhexidine may be beneficial as an adjunct to mechanical debridement, alternative decontamination approaches might be more suitable for the comprehensive treatment of peri-implantitis.

The differential susceptibility of bacterial species to decontamination methods observed in our study has important clinical implications. *P. gingivalis*, a key periodontal pathogen strongly associated with peri-implantitis, showed high susceptibility to all tested methods, particularly Er:YAG laser and photodynamic therapy. In contrast, *S. mitis*, representing early colonizers in biofilm formation, demonstrated greater resistance to chlorhexidine. These findings support the notion that multispecies biofilms may require a combination of approaches for effective decontamination, targeting both early and late colonizers in the biofilm structure.

Surface topography plays a crucial role in subsequent biological responses following implant decontamination. While optimal roughness promotes re-osseointegration, excessive smoothing or surface damage may impair cellular attachment and healing. Our SEM analysis revealed that Er:YAG laser treatment, despite its excellent antimicrobial properties, caused the most pronounced surface alterations. This observation aligns with findings by Schwarz et al., who reported that Er:YAG laser irradiation at certain parameters could result in surface melting and crack formation[5]. In contrast, photodynamic therapy preserved surface characteristics while achieving comparable bacterial reduction, suggesting it may be preferable from a surface integrity perspective.

Several limitations of this study should be acknowledged. First, the in vitro nature of our investigation may not fully reflect the complex in vivo environment of peri-implantitis lesions, which involves host immune responses, varying oxygen tensions, and gingival crevicular fluid components. Second, our biofilm model, while incorporating key peri-implantitis-associated pathogens, represents a simplified version of the complex multispecies biofilms encountered clinically. Third, we evaluated only immediate antimicrobial effects; long-term

outcomes such as biofilm regrowth and surface biocompatibility require further investigation. Finally, the flat titanium discs used in our study do not replicate the complex three-dimensional geometry of dental implants, which may influence treatment efficacy, particularly in areas with limited accessibility[21,22].

5. CONCLUSION

Within the limitations of this in vitro study, Er:YAG laser treatment and photodynamic therapy demonstrated superior antimicrobial efficacy on biofilm-contaminated titanium surfaces compared to air polishing and chlorhexidine irrigation. However, Er:YAG laser caused more pronounced surface alterations, while photodynamic therapy preserved surface integrity while achieving comparable bacterial reduction. These findings suggest that photodynamic therapy may offer an optimal balance between antimicrobial efficacy and preservation of implant surface properties for the management of peri-implantitis. Clinical studies are needed to validate these findings and establish evidence-based protocols for implant surface decontamination in peri-implantitis treatment.

DECLARATIONS

Ethical approval and consent to participate

Not Applicable

Availability of data and material

All data generated or analyzed during this study are included in the published article.

Competing interest

The authors declare that there are no competing interests.

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ORIGINAL RESEARCH

COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF GRAPHENE OXIDE AND REDUCED GRAPHENE OXIDE NANOPARTICLES AGAINST COMMON ORAL PATHOGENS:IN VITRO STUDY

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ABSTRACT

Background: The escalating threat of antimicrobial resistance (AMR) has driven the search for alternative antibacterial agents. Graphene-based nanomaterials, particularly graphene oxide (GO) and reduced graphene oxide (rGO), have emerged as promising candidates due to their unique physicochemical properties and potential antimicrobial mechanisms.

Objective: This study aims to evaluate and compare the antimicrobial efficacy of GO and rGO nanoparticles against common oral pathogens—*Streptococcus mutans*, *Enterococcus faecalis*, and *Candida albicans*.

Materials and Methods: The antimicrobial activity of GO and rGO was assessed via agar well diffusion and time-kill kinetics. GO and rGO were tested at concentrations of 25, 50, and 100 µg/mL, with standard antibiotics as controls. Inhibition zones were measured, and statistical significance was evaluated using one-way ANOVA and Tukey's post hoc test

Results: GO demonstrated superior, dose-dependent antimicrobial activity compared to reduced GO, particularly against *Candida albicans* (18.48 ± 0.06 mm at 100 µg/mL), followed by *S. mutans* and *E. faecalis*. rGO showed limited efficacy in inhibition zone assays, although time-kill kinetics revealed its bactericidal potential, especially against *E. faecalis*.

Conclusion: GO exhibited stronger antimicrobial performance than rGO, likely due to its higher oxygen content and associated reactive oxygen species (ROS) generation. While rGO was less effective in static assays, its performance in time-kill kinetics suggests potential in dynamic or prolonged-contact applications. Graphene-based nanomaterials show promise for dental antimicrobial use, but require further optimization and in vivo validation

Keywords: Graphene oxide, Reduced graphene oxide, Antimicrobial efficacy, Oral pathogens, Nanoparticles

1. INTRODUCTION

The surge in bacterial infections has become a major threat to global health, with significant impacts on public well-being and millions of individuals worldwide. Antibacterial agents are essential for protecting human health from these severe and potentially life-threatening diseases. Nonetheless, the inappropriate and excessive use of conventional antibiotics has led to the emergence of antibiotic-resistant bacteria, rendering many infections difficult, if not impossible, to treat effectively[1]. This phenomenon, known as antimicrobial resistance (AMR), has emerged as one of the most critical and pressing public health challenges of the 21st century[2]. AMR refers to the capacity of microorganisms—including bacteria, viruses, fungi, and parasites—to resist the effects of drugs that were previously effective in eradicating

or controlling infections[3].

The antimicrobial effectiveness of dental products is crucial for fighting oral pathogens, preventing dental caries, gingivitis, and periodontal diseases, while also reducing plaque buildup and controlling bacterial growth to promote overall oral health[4].Various antibacterial materials, including metal ions and oxides, antibiotics, quaternary ammonium compounds, and antimicrobial peptides, have been utilized in efforts to combat bacterial infections. However, each of these materials has its own set of limitations and challenges. Metal ions and oxides are known for their broad-spectrum antimicrobial activity against a range of pathogens, including fungi, viruses, and bacteria. Despite their effectiveness, they can exhibit toxicity towards certain mammalian cells. Prolonged use of antibiotics and quaternary ammonium compounds often leads to the development of resistant strains of bacteria. Furthermore, the production and use of pure antimicrobial peptides as antibacterial agents are often hindered by their high costs. To overcome these challenges, researchers have increasingly turned to alternative antibacterial materials such as metal nanoparticles (NPs), carbon nanotubes, metal oxide NPs, and graphene-based materials[1].

Nanomaterials, defined by dimensions ranging from 1 to 100 nm, display distinct physical, chemical, and biological properties that differentiate them from their bulk counterparts[5]. Nanoparticles (NPs) are insoluble particles smaller than 100 nm, and the science of manipulating them is known as nanotechnology. Due to their small size and ability to penetrate biological systems easily, they are widely used in medicine, drug delivery, and dentistry. Their applications are supported by properties such as antimicrobial activity, friction reduction, anti-inflammatory effects, and antioxidant capabilities[6].

Among the myriad of chemical elements, carbon occupies a distinctive and pivotal role. It serves as the fundamental scaffold for biological systems, thanks to its diverse orbital hybridization states which facilitate the formation of various chemical bonds with multiple spatial configurations.

Consequently, carbon exists in several allotropic forms, such as graphite and diamond, and possesses the potential to produce a broad spectrum of nanostructures, including monolayer graphene sheets, single-walled and multi-walled carbon nanotubes, carbon fibers, fullerenes, carbanions, and Nano diamonds[7].

Carbon is a fundamental element with versatile bonding characteristics that enables it to form a variety of allotropes. These carbon-based materials have gained attention for their potential applications due to their remarkable mechanical and biological properties. Carbon-based nanostructures (CNSs), such as fullerenes, carbon nanotubes (CNTs), and various forms of diamond, are especially noteworthy for their wide-ranging applications in fields such as drug delivery, tissue engineering, imaging diagnostics, and cancer therapy[8]. Among the different forms of carbon, graphene is of particular interest because of its exceptional properties[9].In 2004, Geim and colleagues achieved a significant breakthrough by synthesizing a stable monolayer of graphene through mechanical exfoliation techniques. This accomplishment earned Geim and Novoselov the Nobel Prize in Physics in 2010. Since then, graphene has become one of the most extensively studied two-dimensional materials in scientific research. Graphene consists of a single atomic layer of carbon atoms arranged in a hexagonal lattice, with a thickness of only 0.334 nm, making it the thinnest material known. It possesses a range of unique properties, including an extraordinarily high specific surface area (~2600 m^2/g), exceptional electron mobility (200,000 cm^2/Vs), superior thermal conductivity (3000–5000 W/m·K), remarkable optical transparency (97.4%), and outstanding mechanical strength, with a Young's modulus of 1 Tpa[10].Graphene composites are emerging as versatile materials for developing specialized inks with functions like biocompatibility and electrical conductivity, which can enhance 3D and 4D bio printing processes, enabling the creation of dynamic tissue structures that better mimic natural tissue behavior and support regeneration across various types of tissues, including bone and nerve[11]. Graphene oxide (GO) is a derivative of graphene that has been oxidized to introduce various oxygen-containing functional groups, such as carboxylic acids (–COOH), carbonyls (–C=O), and hydroxyls (–OH), on its surface and edges. These functional groups facilitate interactions with biomolecules and contribute to bacterial inactivation through several mechanisms, including membrane stress, oxidative damage, entrapment, and photo-thermal effects. The sharp edges of GO nanosheets can also cause physical damage to bacterial membranes, leading to bacterial death through the leakage of intracellular components[12-14]. Graphene oxide, a single-layer 2D sheet of sp^2-bonded carbon atoms in a honeycomb pattern, is valued for its strong mechanical properties, electrical conductivity, and especially its barrier capabilities. Its simple synthesis process also makes it an effective carrier in nanocomposites[15]. Another noteworthy derivative of graphene is reduced graphene oxide (rGO). This material is produced by removing the oxygenated functional groups from graphene oxide through electrochemical reduction. The resulting rGO films have a significantly lower number of oxygen-containing groups compared to GO[16].

Due to the distinct differences in their physical, chemical, structural, and electronic properties, graphene (Gr), GO, and rGO interact with bacterial cells in varied ways[17].The rising demand for graphene oxide (GO) and reduced graphene oxide (rGO) in drug delivery, biomedical applications, and diagnostics is largely attributed to their significant impact on cytotoxicity, biodistribution, and biotransformation, with 3D scaffolds of these materials enhancing preclinical research due to their reactive oxygen functional groups and electrical insulation properties[18].

This study evaluates and compares the antimicrobial efficacy of graphene oxide (GO) and reduced graphene oxide (rGO) nanoparticle against common oral pathogens, including *Streptococcus mutans*, *Enterococcus faecalis*, and *Candida albicans*.

2. MATERIAL AND METHODS

Antimicrobial Activity Assessment

Preparation of nano particle

Graphene oxide (GO) nanoparticles were synthesized using a modified Hummers' method. Briefly, graphite powder was oxidized using concentrated sulfuric acid (H_2SO_4), sodium nitrate ($NaNO_3$), and potassium permanganate ($KMnO_4$), followed by controlled heating, washing with distilled water, and centrifugation until a neutral pH was reached. The obtained GO was dried at 60 °C. Reduced graphene oxide (rGO) was subsequently synthesized by chemical reduction of the prepared GO using ascorbic acid as the reducing agent under mild heating and continuous stirring. Both GO and rGO powders (0.100 g each) were dispersed in 5 mL of ethanol, and a series of different concentrations were prepared for subsequent antimicrobial analysis.

The antimicrobial properties of graphene oxide (GO) and reduced graphene oxide (rGO) nanoparticles were assessed using the agar well diffusion method. Mueller Hinton agar plates were prepared, sterilized at 121°C for 15–20 minutes, and then poured into sterile Petri dishes to cool to room temperature. A suspension containing *Streptococcus mutans*,Entercoccus faecalis , and *Candida albicans* was spread across the agar surface with sterile cotton swabs. Wells with a 9 mm diameter were formed in the agar plates using a sterile polystyrene tip and filled with various concentrations of GO and rGO (25, 50, and 100 µg/ml). An antibiotic control (Amoxyrite for bacteria and comparison. Plates were incubated at 37°C for 24 hours (48 hours for fungi), and inhibition zones were measured Fluconazole for fungi) was included for in millimeters (mm) for a comprehensive analysis,

Time-Kill Kinetic Analysis

To further investigate bactericidal effects, a time-kill curve assay was conducted. Pathogens were cultured in Mueller Hinton Broth with varying concentrations of GO and rGO (25, 50, and 100 µg/mL). An antibiotic control was used for comparison, and pre-incubation for four hours allowed pathogens to reach a stable growth phase. A 0.5 McFarland standard inoculum was prepared from Mueller Hinton agar cultures grown at 37°C for 18–20 hours. Thirty microliters of inoculum was diluted into 15 mL of antimicrobial-free broth pre-warmed to 37°C, and 90 µL of this mixture was added to each well of a 96-well ELISA plate. Ten microliters of GO and rGO at five concentrations were added, along with an untreated control, providing insights into antimicrobial kinetics and nanoparticle effectiveness against pathogens.

Statistical Analysis

All experimental data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to determine pairwise differences between groups. A p-value < 0.05 was considered statistically significant. Results are presented as mean ± standard deviation (SD).

3. RESULTS

Graphene oxide (GO) and reduced graphene oxide (rGO) demonstrated varied antimicrobial activities across concentrations and pathogens (Figure 1) .GO exhibited a dose-dependent increase in the zone of inhibition, particularly effective against Candida albicans (18.48 ± 0.06 mm at 100 µg/mL), followed by Streptococcus mutans (11.57 ± 0.05 mm) and Enterococcus faecalis (9.17 ± 0.05 mm).(Table 1)

In contrast, rGO showed limited antimicrobial efficacy with less pronounced differences across concentrations. The highest zone of inhibition was observed against Candida albicans (14.83 ± 0.06 mm at 100 µg/mL), while E. faecalis and S. mutans showed zones below 9.3 mm.(Table2)

Post hoc test revealed significant differences ($p < 0.001$) in antimicrobial activity among concentrations for all organisms tested. GO showed significant differences between all concentrations for each organism. Post hoc tests also confirmed that GO's efficacy, while significant, was consistently lower than the standard antimicrobial agents. (Table 3)

For rGO, while Candida albicans showed statistically significant dose-dependent efficacy, no significant difference was observed between concentrations against S. mutans. The efficacy of rGO was also consistently inferior to the standard controls.(Table 4)

Table 1. Mean Zones of Inhibition Graphene Oxide

Microorganism	Concentration (mg/mL)	N	Mean	SD
E. faecalis	100	4	9.17	0.0520
	25	4	8.88	0.0485
	50	4	9.53	0.0490
	Standard	4	32.08	0.0540
S. mutans	100	4	11.57	0.0530
	25	4	8.67	0.0470
	50	4	9.72	0.0510
	Standard	4	19.38	0.0560
C. albicans	100	4	18.48	0.0590
	25	4	11.07	0.0505
	50	4	11.22	0.0515
	Standard	4	32.48	0.0580

Table 2. Mean Zones of Inhibition reduced graphene oxide

Microorganism	Concentration (mg/mL)	N	Mean	SD
E. faecalis	100	4	8.72	0.0520
	25	4	9.28	0.0495
	50	4	8.67	0.0510
	Standard	4	33.73	0.0550
S. mutans	100	4	8.93	0.0535
	25	4	8.78	0.0480
	50	4	9.03	0.0505
	Standard	4	28.82	0.0570
C. albicans	100	4	14.83	0.0585
	25	4	9.13	0.0515
	50	4	8.63	0.0490
	Standard	4	34.92	0.0600

Table 3. Graphene Oxide (GO) - Post Hoc Comparisons

Microorganism	Comparison	Mean Difference	P Value	Significance
E. faecalis	100 vs 25	0.29	<0.05	Significant
	100 vs 50	-0.36	<0.05	Significant
	100 vs Standard	-22.91	<0.001	Significant
	25 vs 50	-0.65	<0.05	Significant
	25 vs Standard	-23.20	<0.001	Significant
	50 vs Standard	-22.55	<0.001	Significant
S. mutans	100 vs 25	2.90	<0.001	Significant
	100 vs 50	1.85	<0.05	Significant
	100 vs Standard	-7.81	<0.001	Significant
	25 vs 50	-1.05	<0.05	Significant
	25 vs Standard	-10.71	<0.001	Significant
	50 vs Standard	-9.66	<0.001	Significant
C. albicans	100 vs 25	7.41	<0.001	Significant
	100 vs 50	7.26	<0.001	Significant
	100 vs Standard	-14.00	<0.001	Significant
	25 vs 50	-0.15	>0.05	Not Significant
	25 vs Standard	-18.85	<0.001	Significant
	50 vs Standard	-18.70	<0.001	Significant

Table 4. Reduced Graphene Oxide (RGO) - Post Hoc Comparisons

Microorganism	Comparison	Mean Difference	P Value	Significance
E. faecalis	100 vs 25	-0.56	<0.05	Significant
	100 vs 50	0.05	>0.05	Not Significant
	100 vs Standard	-25.01	<0.001	Significant
	25 vs 50	0.61	<0.05	Significant
	25 vs Standard	-25.45	<0.001	Significant
	50 vs Standard	-26.06	<0.001	Significant
S. mutans	100 vs 25	0.15	>0.05	Not Significant
	100 vs 50	-0.10	>0.05	Not Significant
	100 vs Standard	-19.89	<0.001	Significant
	25 vs 50	-0.25	>0.05	Not Significant

The time-kill assay showed that GO had bacteriostatic effects, producing only slight CFU reductions over time. In contrast, rGO displayed a stronger bactericidal trend, especially at higher concentrations. This was most notable in E. faecalis and C. albicans, where CFU counts significantly declined over the 24-hour period, supporting the potential for time-dependent antimicrobial action. (Figure 2).

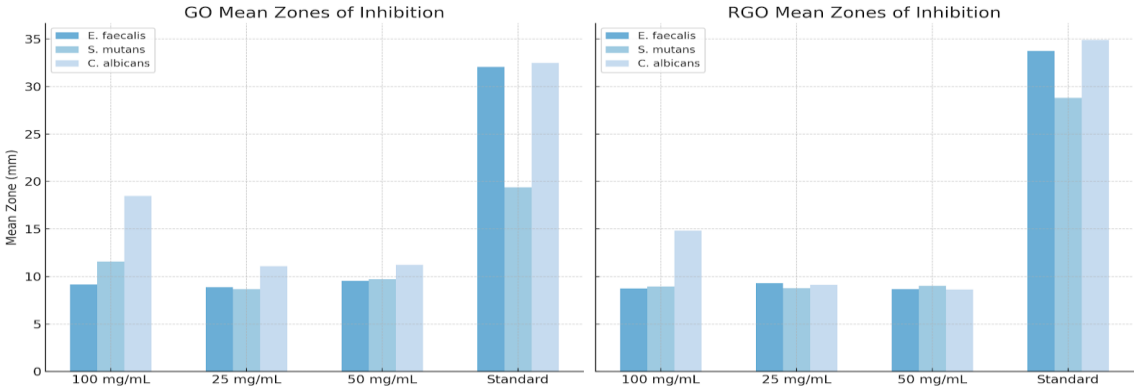


Figure 1. Mean Zones of Inhibition of Graphene Oxide and Reduced Graphene Oxide

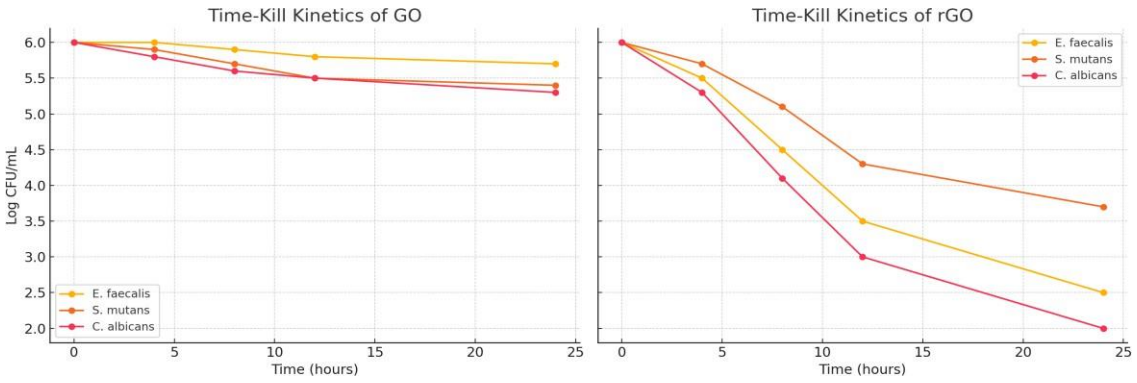


Figure 2. Time-Kill Kinetics of GO and rGO against E. faecalis, S. mutans, and C. albicans over 24 hours.

4. DISCUSSION

The results of the study shows that Graphene oxide demonstrated superior antimicrobial activity, particularly against *Candida albicans*. The zone of inhibition increased in a dose-dependent manner, with the highest concentration (100 µg/mL) producing a zone of 18.48 mm. This enhanced performance of GO can be attributed to its abundant oxygen-containing functional groups, such as hydroxyl and carboxyl groups, which generate reactive oxygen species (ROS) capable of damaging microbial membranes[14]. Furthermore, the time-kill kinetics analysis confirmed that GO has a significant fungicidal effect, reducing the colony-forming units (CFU) of *Candida albicans* over time, particularly at higher concentrations.

On the other hand, reduced graphene oxide showed limited antimicrobial activity compared to GO. The zone of inhibition for rGO remained small for both *Streptococcus mutans* and *Enterococcus faecalis*, with a notable exception at 100 µg/mL against *Candida albicans*, where the zone of inhibition increased to l4.83 mm. The reduced antimicrobial efficacy of rGO can be explained by its lower oxygen content, which reduces the ability to produce ROS and weakens its interaction with bacterial cell membranes [19]. However, despite its weaker performance in the inhibition zone assays, rGO showed stronger bactericidal effects in time-kill kinetics, particularly against *Enterococcus faecalis*. This suggests that rGO may still be effective in dynamic environments where prolonged exposure enhances its bactericidal capabilities.

The observed difference in antimicrobial activity between GO and rGO aligns with existing research. GO's higher oxygen content enhances its ability to generate ROS, causing oxidative damage and physical disruption of bacterial membranes, leading to cell death (Perreault et al.)[20]. These mechanisms explain the greater inhibition zones observed for GO in this study, particularly at higher concentrations. Additionally, the sharp edges of GO nanosheets contribute to membrane piercing, which further enhances its antimicrobial action [12].Reduced graphene oxide's limited efficacy in the inhibition zone assays, particularly against *Streptococcus mutans* and *Enterococcus faecalis*, is consistent with findings that rGO's lower oxygen content diminishes its ROS production, resulting in weaker interactions with bacterial cells (Akhavan et al.).[12]However, the time-kill kinetics data suggest that rGO's antimicrobial action may be more pronounced under prolonged exposure, as seen in its bactericidal effect against *Enterococcus faecalis* at higher concentrations. This indicates that while rGO maynot perform as effectively as GO in static assays, it could still be a viable option in applications where prolonged contact time is feasible.

Despite these promising results, it is important to note that both GO and rGO exhibited lower antimicrobial activity compared to standard antibiotics used as controls in the study. This underscores the challenge of developing nanomaterials that can match or exceed the efficacy of traditional antibiotics. The standard antimicrobial agents showed significantly larger inhibition zones, particularly against *Enterococcus faecalis*, suggesting that further modification or combination of GO and rGO with other antimicrobial agents may be necessary to enhance their effectiveness (Liu et al.)[14]. Combining graphene-based materials with metal nanoparticles or antimicrobial peptides could provide a synergistic effect, improving their bactericidal and fungicidal properties while maintaining their biocompatibility[19].

This study highlights the potential of graphene-based materials, particularly graphene oxide, as antimicrobial agents against oral pathogens. GO demonstrated superior antimicrobial efficacy compared to rGO, particularly against *Candida albicans*, due to its oxygen-rich structure and ability to generate ROS. While rGO showed limited efficacy in inhibition zone assays, its bactericidal properties in time-kill kinetics suggest potential applications in prolonged exposure settings. However, both materials need further enhancement to compete with conventional antibiotics, and future research should explore combining them with other antimicrobial agents or modifying their surface properties to optimize their efficacy.

This study's primary innovation is its direct comparison of graphene oxide (GO) and reduced graphene oxide (rGO) against a diverse range of dental pathogens, enhancing our understanding of their distinct antimicrobial properties. Unlike previous research, which has typically concentrated on either bacterial or fungal pathogens, this study evaluates both simultaneously, offering a more comprehensive assessment. It also emphasizes that while rGO exhibits enhanced properties in other biomedical applications, this does not necessarily equate to superior antimicrobial efficacy against fungal pathogens like *Candida albicans*. Despite these valuable insights, the study has several limitations. The in vitro nature does not replicate the complex interactions found in the oral cavity, where factors like saliva, biofilm formation, and host immune responses are crucial.

Additionally, the study relies solely on inhibition zones to measure antimicrobial activity and did not assess other important aspects such as biofilm prevention, resistance development, and the cytotoxicity which refers to the quality of being damaging to cells[21]. These parameters are crucial for determining their safety in clinical applications.

5. CONCLUSION

Graphene oxide (GO) showed stronger antimicrobial effects than reduced graphene oxide (rGO), especially against *Candida albicans*, due to its oxygen-rich surface and ROS generation. While rGO was less effective in static tests, it demonstrated time-dependent bactericidal activity against *Enterococcus faecalis*. These results support the potential of graphene-based nanomaterials in dental antimicrobial applications, though further in vivo evaluation are needed . Both nanomaterials require further optimization, and their clinical application warrants in vivo validation.

DECLARATIONS

Ethical approval and consent to participate

Not Applicable

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Competing and Conflicting Interests

The authors declare no competing or conflicting interests related to this study.

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RESEARCH ARTICLE

EFFECTIVENESS OF NSAIDS AND TCA IN THE MANAGEMENT OF TEMPOROMANDIBULAR DISORDERS-RETROSPECTIVE STUDY

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ABSTRACT

Purpose: Temporomandibular Disorders (TMD) encompasses a group of disorders involving joint - disc disorders, masticatory muscles and degenerative joint disorders. It is important to use a management strategy that is safe, economical, and effective for patients. While surgical approaches are hindered by certain complications, non-invasive therapies offer these benefits and are therefore considered the first line of treatment for TMDs. Existing literature suggests a lacuna of studies analyzing the effect of Tricyclic Antidepressants (TCA) on TMDs in comparison to Non-Steroidal Anti-inflammatory Drugs (NSAIDs). The aim of this study was to assess the effectiveness of NSAIDs and TCA in managing Temporomandibular Disorders.

Materials and Methods: Data of diagnosed TMD patients who were treated during the time span between January 2022 and January 2024, was retrieved retrospectively from the institutional electronic database. 50 patients were included, and they were divided up into two groups based on the treatment protocol followed. Patients in Group A were managed with NSAIDs, while patients in Group B were managed with TCAs. Outcome measures including pain relief (VAS score), maximal mouth opening were assessed.
Results: 28% patients treated with TCA showed complete pain reduction and 44% patients showed improved mouth opening compared to NSAID s group which showed 12% reduction in pain and 32% improvement in mouth opening.

Conclusion: TCA was found to be more effective in relieving pain and improved mouth opening compared to NSAIDs when supplemented with physical therapy. TCAs can be a viable therapeutic option for TMDs.

Keywords: TMJ Disorders, Anti-inflammatory agents, Non-steroidal, Antidepressants

1. INTRODUCTION

Temporomandibular joint is formed by the insertion of mandibular condyle into the mandibular fossa of the temporal bone. It is regarded as a ginglymoarthroidal joint, meaning it may move in both

hinge and gliding modes[1]. The two most prevalent types of TMD are pain, including headaches, arthralgia, and myalgia, and conditions related to the TMJ, primarily disc displacements and degenerative diseases[2]. The most common symptoms of temporomandibular disorders include pain, discomfort, TMJ noises and limited or asymmetric mandibular motion[3]. It is usual for pain or discomfort to be restricted to the masticatory muscles, jaw, and TMJ. Common accompanying symptoms are headache, neck pain, tinnitus, dizziness, and ear pain[4,5] Chronic facial pain is one of its defining characteristics which lowers people's quality of life and debilitates them physically and psychologically[6] .It has been extensively established that TMD has a multifaceted etiology[7,8] .

Globally, 34% of people have TMDs (South America 47%, Asia 33%, Europe 29%, North America 26%). The significance of TMDs as a disease entity is shown by the vast quantity of cases in the population[9]. Globally, research has shown that between 10 and 15 % of persons have pain from TMD, and 5 % appear to require treatment. Remarkably, the literature has reported a higher female to male prevalence of roughly 10:1.6[10].

In the majority of TMD cases, conservative therapy is the initial course of treatment. This frequently involves the usage of systemic anti-inflammatory drugs, analgesics, dietary modifications, the creation of occlusal splints, physical therapy[11] and TENS therapy[12] . The effects of NSAIDs, corticosteroids, muscle relaxants, anxiolytics, opioids, and TCA have all been investigated in relation to pharmaceutical therapy, but no definitive findings have been reached. Extended use of NSAIDs has been associated with major side effects such as gastrointestinal, hepatic, renal, and cardiovascular issues[13], therefore the need to explore and validate effective alternatives to NSAIDs for managing TMDs remains a crucial area of research. Recent studies have focused on the effect of TCA in alleviating TMD pain[14] . However, studies comparing the effectiveness of NSAIDs and TCA are lacking.

This present study aimed to assess the effectiveness of Non-Steroidal Anti-inflammatory Drugs and Tricyclic Antidepressants in management of TMDs.

2. MATERIAL AND METHODS

This retrospective cross - sectional study was conducted in the Department of Oral Medicine and Radiology, Saveetha Dental College and Hospitals, Chennai, India using the data of patients clinically and radiographically diagnosed with Temporomandibular Disorders obtained from Institutional Electronic Database from January 2022 – January 2024.

Data of diagnosed TMD patients who were treated with NSAIDs and TCA were retrieved. Inclusion criteria were Patients with Myalgia, Myositis, Internal Derangement, Anterior Disc Displacement with reduction / without reduction. Exclusion criteria were individuals who are allergic to NSAIDs/ TCA, individuals below 18 years of age. A convenient sampling technique was used in the present study. Group A consisted of 25 TMD patients who were managed with Non – Steroidal Anti-inflammatory Drugs (Tab zerodol p – Aceclofenac 100mg & Paracetamol 325mg) and Group B consisted of 25 TMD patients who were treated with Tricyclic Antidepressants (Tab.Tryptomer – Amitriptyline Hydro chlorine I. P10mg). Both the groups were subjected to Physical therapy. These patients were treated for 1 month. The outcome parameters assessed were pain (VAS score) and mouth opening. The collected data were entered in the Microsoft Excel 2016 and analysed with IBM SPSS Statistics for Windows, Version 29.0. (Armonk, NY: IBM Corp). To describe about the data descriptive statistics frequency analysis, percentage analysis was used for categorical variables and the mean & S.D were used for continuous variables. To find the significance in qualitative categorical data Chi-Square test was used. In the above statistical tool, the probability value .05 is considered a significant level.

3. RESULTS

A total of 50 patients were included in this study (n = 25 patients who were treated with NSAIDs and n=25 patients who were treated with TCA). Pain (VAS score) recorded at the first visit showed that both NSAIDs group and TCA group had approximately similar proportions of severe pain cases (32% and 28% respectively). Moderate intensity pain cases were also equally distributed among both the groups (48% and 48% respectively). The proportion of mild pain cases were double in TCA group (24%) compared to NSAIDs group (12%).Two patients in NSAIDs group reported with absence of pain, whereas there were no pain-free cases in TCA group. In the first visit, measurement of mouth opening reveals 56.0% and 52.0% of patients had restricted mouth opening in NSAIDs group and TCA group respectively.

The association of pain intensity and mouth opening with grouping at baseline was statistically insignificant ($p = 0.381$ and $p = 0.094$) implying a random distribution between the groups

Pain (VAS score) recorded at the follow-up showed that complete reduction of pain was seen in

n=3 (12%) and n=7 (28%) in NSAIDs and TCA groups, respectively. No reduction of pain was noted in n=9 (36%) in NSAIDs group and n=2 (8%) in TCA group (figure 1). Similarly , in the follow up improvement in restricted mouth opening was recorded in n=8 (32%) and n=11 (44%), respectively in NSAIDs group and TCA group whereas, n=6(24%) in NSAIDs group and n=2 (8%) in TCA group showed no improvement in mouth opening (figure 2).

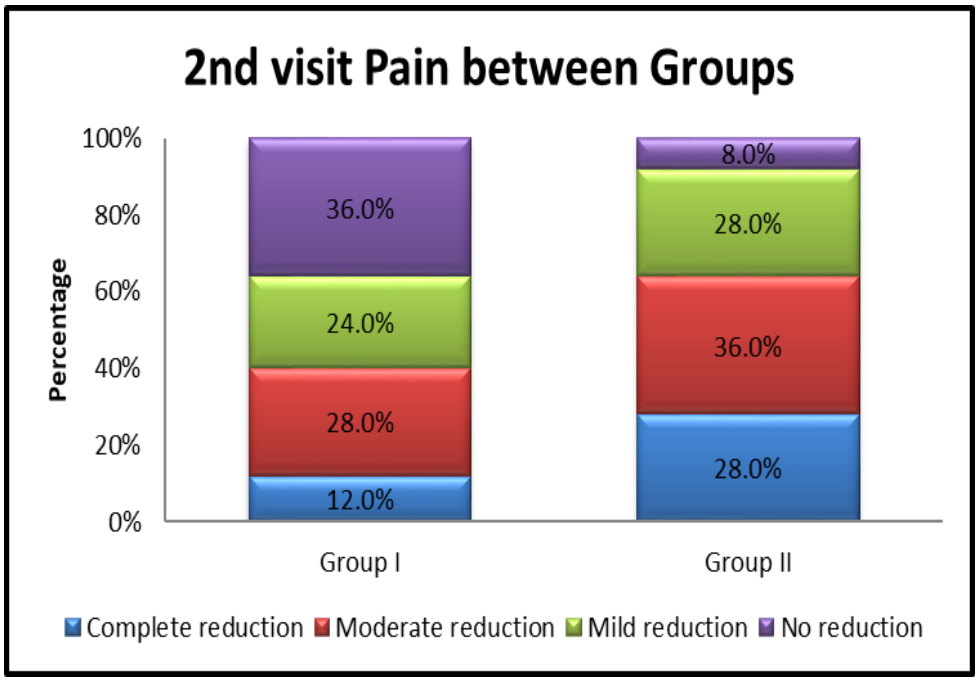


Figure 1. Pain intensity in Group NSAIDs and Group TCA in follow up

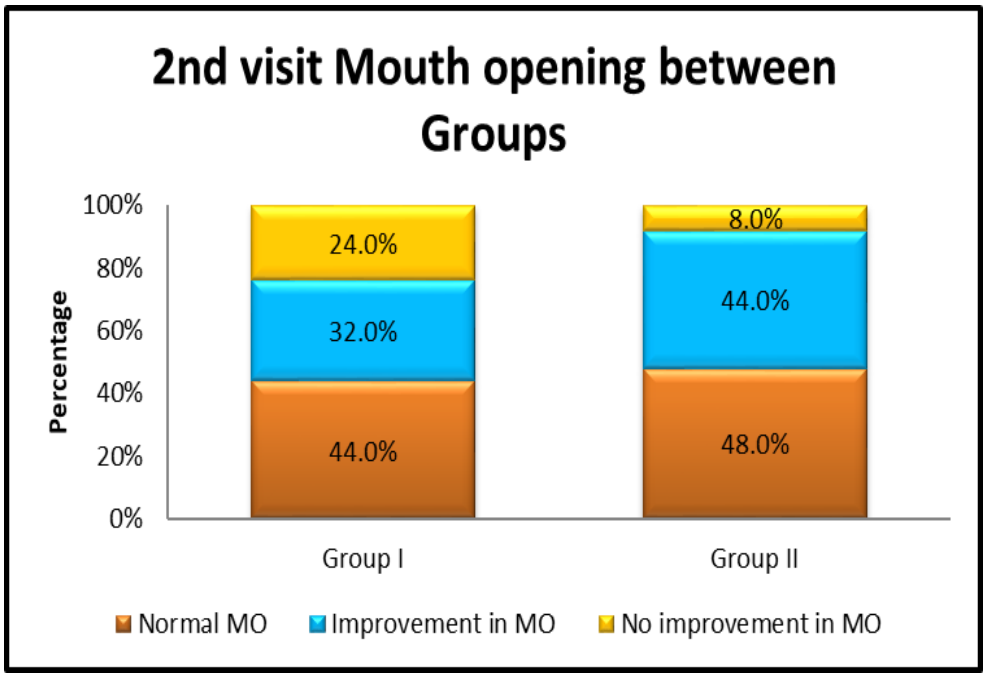


Figure 2. Mouth opening in Group NSAIDs and Group TCA in follow up

At follow-up, it was shown that there was no statistically significant correlation between the two groups' mouth opening and pain intensity (p = 0.094 and p =0.284) **(Table 1, 2).**

TABLE 1. Reduction of pain

			Groups		Total
			Group I	Group II	
2nd visit pain	Complete reduction	Count	3	7	10
		%	12.0%	28.0%	20.0%
	Moderate reduction	Count	7	9	16
		%	28.0%	36.0%	32.0%
	Mild reduction	Count	6	7	13
		%	24.0%	28.0%	26.0%
	No reduction	Count	9	2	11
		%	36.0%	8.0%	22.0%
Total		Count	25	25	501
		%	100.0%	100.0%	100.0%

Pearson Chi-Square, p=0.094

TABLE 2. Improvement in Mouth Opening

			Groups		Total
			Group I	Group II	
2nd Visit Mouth Opening	Normal MO	Count	11	12	23
		%	44.0%	48.0%	46.0%
	Improvement in MO	Count	8	11	19
		%	32.0%	44.0%	38.0%
	No improvement in MO	Count	6	2	8
		%	24.0%	8.0%	16.0%
Total		Count	25	25	50
		%	100.0%	100.0%	100.0%

Pearson Chi-Square, p=0.284

4. DISCUSSION

The term "temporomandibular disorders" (TMDs) refers to pain and dysfunction in the temporomandibular joint (TMJ) and chewing muscles. The most prevalent type of orofacial pain is TMD. TMJ sounds and a restricted range of motion are further indications of TMD. However, the primary motivation for patients with TMDs to seek treatment is persistent discomfort. Patients with TMDs are more likely to have issues with their sleep and tinnitus, and there are reciprocal relationships between TMDs and anxiety and depression.

Pharmacological treatments for TMDs focus on the fundamental pathological processes, with the goal of reducing pain, enhancing joint function, and promoting overall patient wellness.

This study is a retrospective analysis of 50 patients who were managed with NSAIDs (n=25) and TCA (n=25) supplemented with physical therapy. A considerable percentage of the population is impacted by the epidemiology and pathophysiology of temporomandibular disorders, with estimates indicating that 10-15% of individual's experience pain from TMD, and 5% require treatment[15]. There are psychological, structural, and functional components to the complex aetiology of TMDs, which makes therapy more challenging[6]. The high prevalence, particularly among females at a ratio of approximately 10:1.6, highlights the need for effective treatment modalities[2].

This study found complete pain relief was obtained in 3 out of 25 patients (12%) which is in accordance with the study by Yuasa Kurita et al.,[16] which showed 60% improvement in intervention group (NSAIDS and physical therapy) than the control group (untreated) which showed 33% over a course of 4 weeks.

Another study by Di Rienzo Businco et al.,[17] which compared topical and oral NSAIDs showed reduction in pain scores achieving a decrease of 61% in topical group and 59% in oral group highlighting the effectiveness both topical and systemic NSAIDs, but this is in contrast with the study by Marini et al.,[18] in which palmitoylethanolamide (PEA) and NSAIDs are compared and showed statistically significant amount of pain reduction (p=0.0001) and improvement in mouth opening (p=0.22) is seen in PEA group than NSAIDs group and in our study the mouth opening is NSAIDs group is 32%.

The study found that TCAs were associated with a higher percentage of patients reporting complete pain relief (28%) compared to the NSAID group (12%). This is consistent with study by Célia M. Rizzatti et al.,[19] in which there is a significant difference in pain and discomfort in patients who were treated with Amitriptyline 25mg than who were treated with placebo for 14 days. In another study by Plesh et al.,[20] which used Amitriptyline (10-30mg) over a course of 6 weeks for myofascial pain and mixed (myofascial and TMDs) showed improvement in pain reduction at end of 6 weeks and 1-year post treatment.

To the best of the authors' knowledge, no studies have been conducted till date to compare the effectiveness of TCAs with NSAIDs in TMD patients. Calderon et al.,[21] assessed effectiveness of Amitriptyline (TCA) and Cognitive Behaviour Therapy (CBT) in chronic TMD patients which showed combination of CBT and TCA resulted in reduced pain, depression level as well improvement in Quality of Life, sleep. This validates study by Dione et al.,[22] that suggests TCAs address psychological issues like depression and anxiety that accompany TMDs in addition to relieving pain. The ability of TCAs to reduce stress levels may play a crucial role in managing TMD symptoms, as stress is often implicated in the exacerbation of pain[23]. Conversely, NSAIDs, while commonly used due to their anti-inflammatory properties, have been associated with significant adverse effects, particularly when used over an extended period of time[13]. The study's findings underscore the limitations of NSAIDs, particularly while managing chronic pain where alternative therapies like TCAs may be more beneficial. Both treatment groups in this study received physical therapy, which is a critical component in the management of TMDs.

Limitations

The effectiveness of TCAs over NSAIDs, although clinically obvious, was found to be statistically insignificant. This could be attributed to the smaller sample size. However, considering the fact that TCAs are not commonly prescribed for management of TMDs the present study findings can pave way for further research with higher sample size.

Other limitations of the study include the short follow-up duration, retrospective design, individual differences in pain tolerance, psychological factors and adherence to physical therapy, all of which might have influenced the treatment outcomes.

Future Prospects

Larger, prospective, randomised controlled trials with extended follow ups across multicenter to validate the findings, future studies could also assess the risk – benefit ratio by monitoring adverse side effects associated with both drugs. Studies should also explore how individual patient factors such as stress, comorbid conditions and genetic predispositions influence the choice and effectiveness of treatment

5. CONCLUSION

The outcomes of this study show that TCAs(Amitriptyline) particularly in low dose is effective than NSAIDs in pain reduction and improvement in mouth opening when combined with physical therapy. Presently, NSAIDs remain a commonly approached pharmacological intervention due to their anti-inflammatory properties. Nonetheless, their long term usage is associated with systemic side effects, making TCAs a promising alternative. In addition, TCAs are known to address the underlying psychological factors such as stress, anxiety which can exacerbate the symptoms of TMD.

DECLARATIONS

Ethical approval and consent to participate
Not Applicable
Availability of data and material
All data generated or analyzed during this study are included in the published article.

Competing interests
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RESEARCH ARTICLE

REANIMATION OF THE PARALYZED FACE WITH THE TEMPORALIS MYOFASCIAL FLAP TRANSFER

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ABSTRACT

Purpose: This study presents the "Temporalis myofascial flap transfer technique" in reanimating the paralyzed face in five patients afflicted with long standing and irreversible facial paralysis. It is an attempt to highlight the versatility and effectiveness of this method in Facial reanimation of the patient with long standing and irreversible facial paralysis. A thorough review is presented of the methods and techniques available to the surgeon and the study underscores the success of the "Temporalis-myofascial flap transfer" technique in restoring the facial form, function and esthetics of the patient suffering with this debilitating disorder.

Materials and methods: The study was carried out from June 2002 to Oct 2005. A total of 5 cases were selected for the study. Four patients were male and one was female. The age range varied from 20 to 42 years with an average age of 31 years. All surgeries were planned under general anesthesia and a standard protocol was followed for a thorough assessment and evaluation of each case which included a detailed case history, cinical examination including a general examination and examination of the pathosis i.e., facial paralysis and pre and post-operative facial photographs.

Results: The results using the temporalis muscle to reanimate the face were recorded as "excellent", "good", "fair" and "poor" for the eye and mouth separately. The results in all five cases were considered excellent as concerned mouth reanimation and again excellent as concerned eye reanimation in cases 1, 2, 3 and 5. (Only mouth reanimation was done in case 4 as the eye function and symmetry were normal.)

Conclusion: The results of the temporalis-myofascial / temporalis–myofascial–galeal technique are valid, reproducible, and highly successful in reanimating the mouth and the eye. We believe that the temporalis muscle transfer will continue to play an essential role in reanimating the face, particularly for patients with long-standing facial paralysis. Five cases were successfully treated using the Temporalis muscle fascia/galea transposition technique for eye and mouth reanimation. One patient was treated for only mouth reanimation since the eyelid function and eye esthetics were normal.

Keywords: Facial paralysis, Facial reanimation, Myofascial flap, Temporalis muscle transfer, Temporalis myofascial flap

1.INTRODUCTION

The diagnosis of a permanent facial paralysis can be devastating to the patient. Our society's emphasis on physical beauty contributes to this perception and often leads to the isolation of these patients [1]. These patients are plagued by a cosmetic defect and the functional deficits caused by the loss of facial nerve function [2]. This deformity is related to the muscular inactivity on the affected side and the apparent overactivity on the normal side. Lagopthalmos with resulting ocular exposure, loss of oral competence with drooling, alar collapse with nasal airway obstruction and difficulties with mastication and speech production are all the potential consequences of facial paralysis [3].

Various methods are available to treat long-standing facial paralysis. They can be static, such as using eyelid weights, and dynamic, like the muscle transfer procedures, which use local or distant muscles to reanimate the face [4]. The distant muscle transfers, however, involve invasive microvascular surgical procedures for reanimation. Hence, local muscle transfers such as the masseter, platysma, and temporalis are preferred for their ease and reliability in rehabilitating the patient with long-standing facial paralysis [5].

This study presents the "Temporalis myofascial flap transfer technique" in reanimating the paralyzed face in five patients afflicted with long-standing and irreversible facial paralysis. It is an attempt to highlight the versatility and effectiveness of this method in the facial reanimation of patients with long-standing and irreversible facial paralysis [6]. A review is presented of the methods and techniques available to the surgeon, and the study underscores the success of the "Temporalis-myofascial flap transfer" technique in restoring the facial form, function, and esthetics of the patient suffering from this debilitating disorder.

MATERIAL AND METHODS

The study was carried out from June 2002 to August 2005. Five cases were selected for the study. Four patients were male, and one was female. The age range varied from 20 to 42, with an average age of 31.

All surgeries were planned under general anesthesia and the following protocol was followed for a thorough assessment and evaluation of each case which included a detailed case history, clinical examination including a general examination and examination of the pathosis i.e., facial paralysis and pre and post-operative facial photographs.

Patients were evaluated for the following parameters:

- Facial symmetry
- Symmetry of the interpalpebral fissures
- Eye closure
- Presence of lagopthalmos
- Mouth symmetry
- Prominence of the naso-labial fold
- Drooping of the corner of the mouth.
- Deviation of the corner of the mouth on speaking and during the execution of facial movements
- Drooling of saliva or foods and liquids from the mouth during eating, speaking and the execution of mouth movements
- Ability to blow the cheeks
- Any speech disturbances

Based on the history and physical examination findings, a diagnosis of Bell's palsy or permanent facial paralysis was made. All investigations for general anesthesia were done and clearance for general anesthesia was obtained. A total head shave was done for all the male patients, whereas the hair was clip-parted for the female patients.

Case Presentation

Surgical procedure for the "Temporalis-myofascial flap transfer"

Nasal intubation was performed to administer general anaesthesia.

Incision lines were marked using a marking pen. 8cc of 1:300000 saline adrenaline was infiltrated along the planned lines of incision at the right/left hemicoronal, temporal, and pre-auricular region. Using the No.15 Bard-Parker blade, a right/left modified hemicoronal incision was taken extending vertically downward in the temporal region and inferiorly into the right/left pre-auricular skin crease as far as the attachment of the tragus (Figure 1).

Skin flaps and the superficial temporoparietal fascia were raised to expose the deep temporal fascia. In two cases, 1.5-2 cm of epicranium was attached to the temporalis muscle to gain additional length (Figure 2). The muscle was traced down inferiorly deep to the zygomatic arch. Bleeding from the superficial temporal and transverse facial arteries was controlled by clamping and ligation with silk sutures. In four cases for both mouth and eyelid paralysis, tunneling of the skin of the upper and lower eyelids was done on the right/left side by blunt dissection using sharp scissors and artery forceps. The forceps were brought out at the medial end of the eyelids through an incision made near the medial canthus. The patency of the submucosal medial canthal incisions near the medial border of the eyelids and the tunnels for the upper and lower eyelids were maintained with the help of two separate ribbon gauze strips. In all five cases, a lip-cheek crease incision of about 1.5 cm in length was taken at the site of the new nasolabial groove (to be reconstructed) in a slightly more medial position than the final result was to be achieved (Figure 1). The location of this incision was identified by placing a finger at the corner of the mouth on the operative side and lifting the lips to create a smile. Soft tissues were undermined from the overlying orbicularis oris muscle to identify the muco-cutaneous junction. The incision was extended through the skin, submucosa and some part of the orbicularis oris muscle. A wider tunnel of two finger breadth was created from the superior preauricular incision starting from the root of the zygoma in the subdermal plane connecting with the lip-cheek crease incision at the nasolabial groove (Figures 3, 4, 5). Another tunnel was created from the nasolabial incision medially across the upper lip to a vertical stab incision just lateral to the ipsilateral philtral column. Patency of these tunnels was maintained with roller ribbon guaze. Temporalis muscle was divided into one/three/four slips pertaining to the individual case (fig. 2, 3). Length of the muscle slips in relation to the area of insertion were checked. Distance between the root of Zygoma and commissure of lip at the right/left side was measured. The slips extended down to the level of the zygomatic arch along the long axis starting from the epicranial attachment. In the case involving only the mouth, the temporalis musle was divided into just a single, broad central slip. Fascial layer was sutured with the individual muscle slips with 3-0 vicryl sutures along the superior edge and few sutures on either side for reinforcement. Muscle slips were elevated by separating the epicranium and periosteum from the underlying bone. Depending on the required measurement for length to reach the corner of the mouth, lengthening of the posterior (3rd slip) was done by sagitally dividing the muscle. Epicranial attachment was sutured to the muscle slips in two cases in which more additional length was needed.

Insertion of the muscle slips (Figure 6, 7, 8): The anterior (first) slip was rotated medially and inferiorly in such a manner that the fascial layer faced the dermal layer of the tunnel and was inserted into the tunnel created at the upper eyelid and was brought out medially through the stab incision and was secured with the dermal layer and orbicularis oculi muscle at the medial canthal region using 3-0 prolene sutures. In a similar fashion the middle (second) slip was inserted through the lower eyelid tunnel upto two thirds the length and was secured with the dermal layer using 3-0 prolene sutures. Another tunnel was created along the medial border of the eye connecting the previous two stab incisions at the medial end of the upper and lower eyelids. Excess length of tissue after securing the anterior slip at the medial end of the upper eyelid was inserted through the tunnel created and was brought down and secured with the middle slip at the lower eyelid using 3-0 Prolene sutures. Posterior (third slip) was divided through and through along the long axis involving the fascial layer at its free end for about a length of 2.5cm creating a bifurcation at its anterior end. Entire posterior slip along with it’s bifurcated ends was rotated medially over the zygomatic arch and inserted into the pre auricular tunnel and was brought out through the lip crease incision. The upper bifurcated The end was secured with the orbicularis muscle using 2-0 Prolene. After securing the excess length, it was inserted into the tunnel created at the upper lip region, brought out at the opposite philtrum, and secured using 2-0 Prolene with the dermal layer.In the cases involving only paralysis of the mouth, a single

central slip of the temporalis muscle and fascia was used and passed through the tunnel above the zygomatic arch (Figure 8).

The lower bifurcated slip was secured with 2-0 prolene sutures just 2 cm above the commissure and was slinged around a layer of orbicularis oris muscle, brought above and backward and was sutured with the dermal layer of the tunnel involving a part of superficial skin using 2-0 prolene thereby overcorrecting the defect and creating a perpetual smile or dimple in the over corrected position. The lip crease incision was closed by subcuticular sutures using 3-0 vicryl. Remaining incisions (at philtrum and medial canthal region).We were closed by interrupted sutures using 4-0 Vicryl. No.10 mini vac drain was placed at the defect created after elevation ofthe temporalis muscle and was secured extraorally with silk sutures in the pre-auricular region. Hemicoronal incision with its pre-auricular extension was sutured in two layers using Vicryl and black silk sutures. Post-operative recovery of the patients was well-monitored and was uneventful.

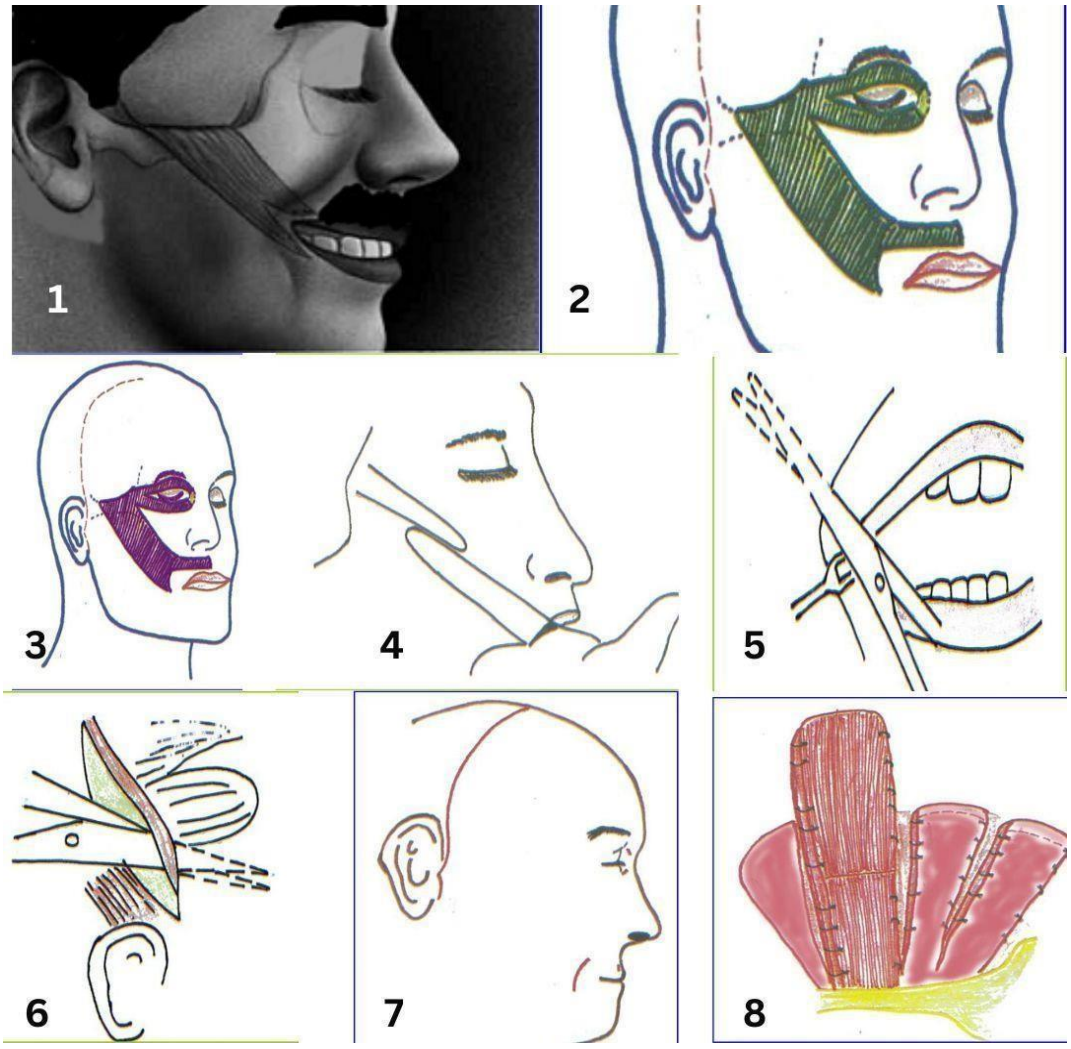


Figure 1-8. Illustration showing stepwise facial dissection techniques including incision planning, muscle exposure, flap elevation, intraoral access, and layered closure for surgical access to facial musculature.

RESULTS

The results of using the temporalis muscle to reanimate the face were recorded as “excellent,” “good,” “fair,” and “poor” for the eye and mouth separately.

The results for **Eye Reanimation** were considered:

Excellent: when the patient could achieve complete approximation of the paralyzed eyelids with attempts to close the eye.

Good: If a slightly incomplete approximation of the eyelids was achieved with just a 1-2mm gap remaining.

Fair: If a poor approximation of the eyelids was achieved, but symmetry of the eyelids was present in the resting open position

Poor: If the interpalpebral fissure was wider on the paralyzed side than on the normal side.

Mouth Reanimation

Criteria for judging the success of this procedure include the achievement of mouth symmetry and a voluntary smile. The results of transposing the temporalis muscle to reanimate the mouth were evident by 3 to 6 weeks after the procedure, although improvement continued for as long as a year postoperatively. Because the patient’s learning can enhance the results of muscle transposition to activate the transposed muscle by voluntary efforts, the procedure results were best in patients motivated to learn the necessary motor-sensory co-ordination techniques.

The results of mouth reanimation were considered:

Excellent: The patient could voluntarily create a smile that exposed the teeth.

Good: If the patient could smile without showing the teeth.

Fair: If the corners of the mouth were symmetrical with the lips in repose.

Poor: If the corner on the paralyzed side drooped.

Given the above gradings, the results in all five cases were considered excellent as concerned for mouth reanimation and again excellent as concerned for eye reanimation in cases 1, 2, 3 and 5. (Only mouth reanimation was done in case 4 as the eye function and symmetry were normal.)

The overcorrection that resulted in an upward and lateral displacement of the lateral oral commissure persisted for about three weeks and then decreased so that the position of the oral commissure was only slightly overcorrected by the sixth week . At this time, the muscle spasms began to subside, and the patients began to show dramatic improvement in movements of the mouth and eye .

The patients began to demonstrate eyelid closure, and symmetry of the interpalpebral fissures was obtained (fig.9, 11, 12, 14, 15, 20, 21). Mouth symmetry was restored (Fig. 9, 12, 16, 18, 20). The patients were able to produce a broad smile which showed the teeth by tensing the temporalis muscle (fig.10, 13, 17, 19, 22). The ability to blow the mouth was restored in all cases. Mouth movement and eyelid closure improved for a year in all cases.



Figure 9-12. Preoperative and postoperative images showing patients with facial asymmetry and muscle weakness undergoing surgical correction and rehabilitation, demonstrating improved facial symmetry, oral competence, and muscle tone over time.



Figure 13-16. Sequential postoperative recovery of a patient with facial nerve paralysis, illustrating progressive improvement in facial symmetry, eye closure, oral competence, and muscular function through surgical intervention and rehabilitation.



Figure 17-22. Sequential postoperative recovery of a patient with facial nerve paralysis

DISCUSSION

A patient with facial palsy presents one of the most difficult challenges the reconstructive surgeon encounters. Understanding facial expressions and the physiology of nerve injury and its repair is critical for evaluating and treating this problem. Only after careful discussion with the patient and developing an individualized treatment plan can a satisfactory result be obtained.

Bell's palsy, viral in origin, is by far the most common cause of facial paralysis, representing 75% of the patients in one large series[6].

Documentation of the clinical findings is essential for the work-up, both for diagnostic and prognostic purposes. Photographs are a useful adjunct if taken in a standardized fashion.

The established facial palsy patient has aesthetic and functional goals that must be addressed in the reconstructive plan. In our study **"Reanimation of the paralyzed face with the Temporalis-myofascial flap transfer technique",** out of the five patients with facial paralysis; three patients suffered from long

standing Bell's palsy while one patient suffered with irreversible, post-trauma facial paralysis of more than 1 year duration and one patient sustained with irreversible facial paralysis following cerebello-pontine angle surgery one year back. Two patients suffered from right-sided facial paralysis, and two patients from left-sided facial paralysis involving both the eye and the mouth. One patient had only left-sided mouth palsy. Four patients – three males and one female had paralysis of both the eye and the mouth while all five showed some or all of the following features:

- Facial asymmetry.
- Lagopthalmos with incomplete closure of the eye on the affected side.
- Widening of the interpalpebral fissure on the affected side.
- Drooping of the corner of the mouth on the affected side.
- Drooling of food and liquids from the affected side of the mouth.
- Difficulty in speaking.
- Loss of controlled balance and difficulty in making facial movements.
- An inability to express certain emotions.
- Deviation of the corner of the mouth to the opposite side when an attempt was made to smile or speak.
- Inability to blow the cheeks.

The patient with only mouth palsy had symmetry of the eyes with equal width of the interpalpebral fissure on both sides. Also, eye closure was adequate in this case and there was no evidence of lagophthalmos.

Several studies have been presented on eyelid and mouth reanimation muscle transfer. Regarding muscle transfers, facial reanimation with Temporalis muscle transfer has withstood the test of time and is still a reference technique. Good results can be obtained in a few weeks with a single and relatively simple surgical procedure. There is no current consensus for objectively evaluating postoperative smiling outcomes. Several objective measurement systems have been proposed [7], but no single system has been adopted due to their complexity, cumbersome nature, or cost. With such variation in reporting results, it isn't easy to make comparisons and conclusions about the outcome of different surgical techniques.

Temporalis muscle transpositions are highly successful in long-standing and irreversible facial paralysis. Analysis of the results of some studies has shown the Temporalis muscle transposition technique to be highly successful and the method of choice alone in long-standing and irreversible facial paralysis or to augment the effects of facial nerve grafting or hypoglossal-facial nerve anastomosis in reanimating the mouth. For patients with facial palsy, lagophthalmos is often a more serious problem than the inability to smile. Dynamic reconstruction of eye closure by muscle transposition or free functional muscle transplantation offers a good solution for regaining near-normal eye protection without needing implants. Transposing the Temporalis muscle and suturing it to the submucosa or periorbicularis oris muscle in the region of the angle of the mouth can restore a voluntary smile and facial symmetry to patients with long-standing facial paralysis. It has also been found that it is usually not necessary to transpose the masseter muscle since excellent results have been achieved with the Temporalis muscle alone. For facial reanimation, the Temporalis muscle has enjoyed more popularity than the masseter because of its position, its facility for greater excursion of movement, and its adaptability to the orbicularis oris.

Advantages of the temporalis muscle flap procedure are multifold:

- The temporalis muscle can be lengthened in continuity of it's natural galea attachment.
- The reconstructive procedure avoids additional injury to the facial nerve.
- As with other temporalis muscle transfer techniques the direction of the muscle pull is proper. The temporalis muscle with galea will pull up laterally on the mouth to evoke a smile and across the eyelids to close the palpebral fissure.
- Theoretically, a patient can learn to operate one muscle independently of the other i.e., the eye can be closed without the appearance of a smile and a smile can be achieved without the eyes being closed.
- Tissue bulk to the cheek when facial tissues are missing or when they have

- atrophied from a prolonged standing facial paralysis is provided.
- Direct muscular insertion in the structures to be moved is achieved.

Disadvantages of the temporalis muscle flap procedure:

- Motion has to be triggered by a muscle of mastication rather than by one of facial expression.
- The motion has to be learned and much time spent in education to achieve effective mobility.
- Emotion and feelings have to be recognized by the patient and then voluntarily transferred to a facial expression.
- The patient has to learn to reduce facial movements during chewing and clenching of teeth.
- The described procedure does not make use of myoneurotization. Muscle interdigitation is not possible and it is unlikely that the nerve to the temporalismuscle will cross the galea to reach the denervated facial muscle. The procedure however does not preclude normal crossover or cross nerve grafts with muscle implantation from the normal side.

- In developing Galea flaps the surgeon has to be careful not to damage the hair follicles of the scalp. Scalp dissection is difficult and hair loss is a possibility.
- Patient's appearance can also be affected by the bulging of muscle over the zygomatic arch and by the post operative hollow in the temporal region if it is not replaced by a suitable substitute.

The other muscles that are commonly used for facial reanimation include the masseter transfer first delineated by Lexer [8], pectoralis minor vascularized muscle graft, extensor digitorum brevis, Gracilis, latissimus dorsi, rectus abdominis, Palmaris Longus tendon, and platysma. Facial reanimation using CFNG results in spontaneity and synchronicity with the healthy side. Additional simultaneous masseteric coaptation allows for fast and powerful reinnervation. This dual innervation technique combines the benefits, resulting in powerful and coordinated facial movements [9]. Temporalis tendon transfer is a relatively straightforward procedure with distinct advantages compared to other forms of facial reanimation, and it provides outstanding results.

Other methods of dynamic facial reanimation include direct nerve repair and grafting, cross-face nerve grafting, and nerve crossovers. They play a role in treating facial paralysis of short-term duration and, as such, are not of much relevance to the cases included in our study. The masseter-to-facial nerve transfer is also an effective method for reanimation of the midface and perioral region in a select group of facial paralysis patients. The technique is advocated for its limited donor-site morbidity, avoidance of interposition nerve grafts, and potential for cerebral adaptation, producing a strong, potentially effortless smile [10]. Using a minimally invasive approach, the temporalis tendon can also be transposed for immediate dynamic reanimation of the paralyzed lower face. This procedure involves a single small incision and minimal dissection, with no foremost osteotomies. Also new software such as FACE [11] has been introduced for the analysis and evaluation of facial function but is not very popular as of now. insertion, and suturing fascia lata to it.

In our study in order to achieve extra length, we used the temporalis fascia in one case by lifting it off the muscle and reinforcing it by suturing it at the end and it's margins for lengthening the temporalis sling. Galea was also used in two cases in an attempt to gain additional length for the temporalis sling to reach the corner of the mouth. However in four of our cases, the muscle was sagitally split wherever required in it's upper third portion to gain the extra length required to reach the paralyzed corner of the mouth. By this innovative method, we were able to obtain a total length of almost 14-15 cms in all three cases and thus, we could very passively secure the temporalis muscle to the orbicularis muscle fibres as well as the dermis both at the oral commissure and at the philtrum. Also, the blood supply to the temporalis was preserved as the muscle was split mid-sagitally. This technique further precluded the use of fascia lata or other modalities to increase the length and was found to be more superior than the use of galea or deep fascia as the muscle itself was

provided to the corner of the mouth thereby enhancing the function of smiling.

In our study, case1, the patient a female, had long hair growth and good volume; the post-op hollow in the temporal region was thus camouflaged. In cases 2,3,4 and 5, the temporalis was found to be thin in volume and the post operative hollow was too miniscule to pose a cosmetic problem. For the same reason the bulk over the zygomatic arch was also not evident.

The years of one sided facial paralysis make for abnormal facial movements with the normal side overwhelming the weakened muscles. A prolonged careful relearning on how to smile is needed. Instructions are given to the patient on how to limit the full excursion on the normal side and how to strengthen the weakened muscles on the paralysed side with the help of the temporalis muscle and how to clench and smile or speak. With frequent sessions in front of the mirror the patient learns how to smile.

All said and done, the temporalis muscle flap remains a versatile and essential tool in plastic reconstructive surgery. Dynamic Facial Reanimation with orthodromic Temporalis tendon has also been tried successfully in children. Temporalis muscle transposition and lengthening myoplasty are also good options for patients who are not good candidates for neurotization by the facial nerve[13]. Chenney et al.[14] has also advocated a modified temporal tendon approach. For restoring a truly spontaneous smile and facial muscle movement, Temporalis muscle transposition can also be used to immediately treat complete facial paralysis due to injuries, leaving the facial nerve anatomically intact but requiring a prolonged recovery time (more than 1 year). Because temporalis muscle transposition does not interfere with neuronal regeneration, it may also be employed early in managing complete facial paralysis when recovery is predicted to be extended and incomplete.

Five cases were successfully treated using the temporalis muscle-fascia / galea transposition technique for eye and mouth reanimation. One patient was treated for only mouth reanimation since the eyelid function and eye esthetics were normal. More than satisfactory results were obtained in all five cases.

CONCLUSION

Facial paralysis is a grave deformity and a setback for the patient in terms of both emotion and function . Of the muscle transfer techniques, the temporalis muscle has shown time-proven results and is quite popular. The results of the temporalis-myofascial / temporalis-myofascial-galeal technique are useful, reproducible and highly successful in reanimating the mouth and the eye. We believe that the temporalis muscle transfer will continue to play an important role in reanimating the face, particularly for patients with long standing facial paralysis. Five cases were successfully treated using the temporalis muscle-fascia / galea transposition technique for eye and mouth reanimation. One patient was treated for only mouth reanimation since the eyelid function and eye esthetics were normal. With our modification of sagittal splitting of the muscle, we gained more than the extra muscle length to reanimate the mouth. This technique described in our study is innovative, most reliable and a safe procedure to gain the extra length as required.

DECLARATIONS

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Ethics approval

Ethical Committee Clearance Number: Not Required

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CASE SERIES

THE ROLE AND SIGNIFICANCE OF MYRIAD MATRIX SOFT TISSUE BIO-SCAFFOLD IN ORAL CAVITY TISSUE RECONSTRUCTION POST -CANCER SURGERY-CASE SERIES

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ABSTRACT

Reconstruction of the soft tissue defects post cancer surgery remains a significant clinical challenge due to the hostile environment of the oral cavity, characterised by polymicrobial colonization, constant movement and presence of high moisture levels persistently. Conventional techniques like the split-thickness grafts and pedicled grafts have been widely used. However, they entail donor site morbidity, prolonged surgical time and variable esthetic and functional outcomes. The Myriad matrix, a decellularized ovine forestomach matrix (OFM), represents a novel biologically active scaffold that supports rapid tissue integration, angiogenesis, and epithelialization. This case series presents two patients with histopathologically confirmed oral squamous cell carcinoma (OSCC) involving the right buccal mucosa and the lateral aspect of the tongue. Both the patients underwent oncologic resection, followed by soft tissue reconstruction using the Myriad matrix soft tissue bio-scaffold. In both the cases, the matrix contributed a stable scaffold for closure, eliminated the need for autologous tissue harvesting, and allowed for complete mucosalization of the defect site. Postoperative follow-up revealed excellent healing, with no evidence of graft-related complications. Functional recovery, including speech, deglutition, and tongue mobility, was well re-established. These findings emphasize the clinical advantages of OFM in oral tissue reconstruction, highlighting its reliability, ease of use, and regenerative potential. The Myriad matrix may thus serve as a valuable alternative in reconstructive strategies, particularly in resource-limited surgical settings.

Keywords*:* Oral squamous cell carcinoma, Soft tissue defect, Wound Healing, Myriad matrix, Ovine Forestomach Matrix, Biocompatible Materials

INTRODUCTION

Reconstruction of soft tissue defects, precipitated by the surgically excised tumorous lesions, present significant challenges to clinicians during management. The oral cavity, in particular, is considered a dynamic and an adversarial habitat owing to the colonization of microbial bioburden, exhibition of perpetual motion and persistently high moisture levels [1]. Oral cancer potentially originate from the squamous cells and later spreads to the other body parts if not intervened promptly. The oral squamous cell carcinoma (OSCC), being the most common malignancy occurring in the head and neck region exhibits an 80% 5-year survival rate at stage I, but drastically drops to merely 15% at stage IV. Management involves either unimodal or multimodal approach encompassing surgery, chemotherapy and radiotherapy. In addition, reconstruction of the post-surgical defect and further oral rehabilitation plays a key role in esthetics and functional outcomes of management strategies [2–4]. Reconstructive techniques traditionally encompasses, split thickness grafts, pedicle grafts or free soft- tissue grafts. However, they entail their own limitations, including extended surgical or operative time, high cost, donor site morbidity, and unpredictable functional and esthetic outcomes [5]. In this respect, several biological scaffolds have been explored for managing such clinical scenarios, and the Myriad matrix soft-tissue bio scaffold, which is the ovine forestomach matrix (OFM) has been widely investigated and has gained immense attention in soft tissue reconstruction [6].

OFM is an extra cellular matrix (ECM) which is the decellularized form, derived from the forestomach of sheep. It consists of matrisomal proteins, retaining its native ECM elements, along with other essential proteins and growth factors such as fibroblast growth factor (FGF), epidermal growth factor (EGF), and connective tissue growth factor (CTGF). These structural elements have been known to play crucial roles in stimulating angiogenesis, regulating inflammatory status, modulating cellular proliferation and differentiation, all of which embrace towards tissue regeneration [7]. The structural properties of OFM, including its high porous nature accelerate its action as a promising bioscaffold for new tissue growth in environments, where the conventional grafts may fail to accomplish adequately [8]. OFM has been successfully integrated in reconstruction of complex soft tissue defects, including those involved with bone exposure or neurovascular bundles [9]. Recent documentations have emphasized the successful utilisation of OFM in the reconstruction of oral cavity defects, especially those involving glossectomies, or excision of growths, involving the buccal mucosa and floor of the mouth. Su *et al.*, have reported successful glossectomy defect reconstruction using OFM grafts. It is considered to possess immense potential in tissue regeneration owing to its ability to rapidly integrate within the surrounding tissue architecture, complete epithelialisation, and minimal or no scarring [10]. In addition, graft related infections or complications have not been observed, even when they are used with chemotherapy. Owing to its superiority over other commercially available ECM products such as cost effectiveness, stability, adaptability to different defect geometries, and ease of handling, OFM has evolved as an invaluable gratuity in the reconstructive spectrum, particularly in the resource-constrained circumstances. The current case series describes the successful management of squamous cell carcinoma of the tongue and the buccal mucosa which were surgical excised and reconstructed with the Myriad soft tissue matrix.

Case description 1.

A 55-year-old male patient reported to the Oral and Maxillofacial Surgery outpatient department at Sri Ramachandra Dental College, Chennai, India, with a primary complaint of an ulcer on the right side of his cheek, which was non healing and was persistent for the past three months. The patient presented that he was in normal health until three months ago when he noticed a sharp tooth in the upper right posterior region. The impingement and irritation from this tooth led to recurrent trauma against the inner aspect of his cheek, resulting in the development of an ulcerative lesion. Over time, this ulcer gradually increased in size, and the patient commenced to experience a burning sensation and continuous, gnawing pain. On general extraoral examination, the patient was conscious, co-operative and oriented. Intraoral examination revealed an ulceroproliferative lesion approximately 4 × 3 cm in size, located on the right buccal mucosa. Anteroposteriorly, the lesion extended about 3 cm from the right oral commissure to the right retromolar trigone. Superoinferiorly, it spanned from 1 cm above the occlusal plane of the right posterior teeth down to the lower mucovestibular region, involving the gingivobuccal sulcus. The lesion appeared erythematous with irregular margins. No bleeding or discharge was observed. On palpation, the ulcer was indurated and mildly tender. There was no clinical evidence of palpable lymphadenopathy. The patient's past medical history revealed a recent diagnosis of hypertension, for which he has not yet commenced any medication. There was no other contributory medical history. There was no relevant past surgical history, or known drug allergies.



Figure 1. 1a. Preoperative profile picture, 1b. Incisional biopsy suture wound of suspected oral squamous cell carcinoma of the right buccal mucosa

An incisional biopsy of the lesion was performed. He was subsequently advised to undergo a contrast-enhanced computed tomography (CECT) scan which identified a subcentimetric right submandibular lymph node, suggesting possible early nodal involvement. The histopathological examination of the biopsy confirmed the presence of a well-differentiated squamous cell carcinoma (SCC) involving the right buccal mucosa. The CECT findings corroborated with this diagnosis and also revealed a right submandibular lymph node measuring less than 1 cm in diameter. The patient has since been admitted for further evaluation and management. Based on clinical, histological, and radiological findings, a final diagnosis of a well-differentiated squamous cell carcinoma of the right buccal mucosa was made.

Surgical management

The patient was brought to the operating room and placed in a supine position. After administration of intravenous general anesthesia, left nasal intubation was performed. The patient was catheterized, and both intraoral and extraoral regions were prepared with 10% povidone-iodine solution. Sterile draping was completed, and markings for a transverse skin crease incision were made.

A skin incision was placed, and subplatysmal dissection was carried out. The sternocleidomastoid (SCM) muscle was exposed and retracted laterally. Dissection was performed along the anterior border of the SCM, allowing identification of the internal jugular vein (IJV). Lymph node dissection was carried out at Levels IIA and III. The spinal accessory nerve (SAN) was traced, identified, and preserved during the procedure. Level IIB lymph nodes were then carefully resected. Dissection continued anteriorly, exposing the anterior and posterior bellies of the digastric muscle. The marginal mandibular nerve was identified and secured. The submandibular gland, along with Level IB lymph nodes, was excised, and the facial artery and vein were ligated. Dissection proceeded into the submental triangle, and Level IA lymph nodes were removed. The surgical area was irrigated thoroughly with saline and betadine, and a Romovac drain was placed.

Teeth, 42, 44, 14, 15, 17, and 18 were extracted. A wide local excision of the lesion involving the right buccal mucosa was performed in conjunction with a marginal mandibulectomy extending from tooth region 42 to 48. A frozen section from the posterior margin was sent for histopathological analysis, which confirmed the absence of malignant cells. Following copious irrigation, intraoral closure was done using the myriad matrix and 3-0 Vicryl sutures. Extraoral closure was completed with 2-0 Vicryl, and skin staples were applied. Final dressing was performed, and the throat pack was removed.

Post-operative care and follow up

Following the procedure, the patient was extubated in a stable condition before being transferred to the Post-Anesthesia Care Unit (PACU). Postoperative care involved effective pain control, strict oral hygiene maintenance,

and scheduled follow-up visits to assess healing progress and minimize potential complications. At the 13-month postoperative followup, the defect site exhibited complete mucosal coverage and excellent healing, with well-integrated buccal tissue and no signs of ulceration, necrosis, or infection. The absence of fibrosis or scarring indicated optimal tissue regeneration, confirming the successful epithelialization and integration of the Ovine Forestomach Matrix (Myriad matrix).

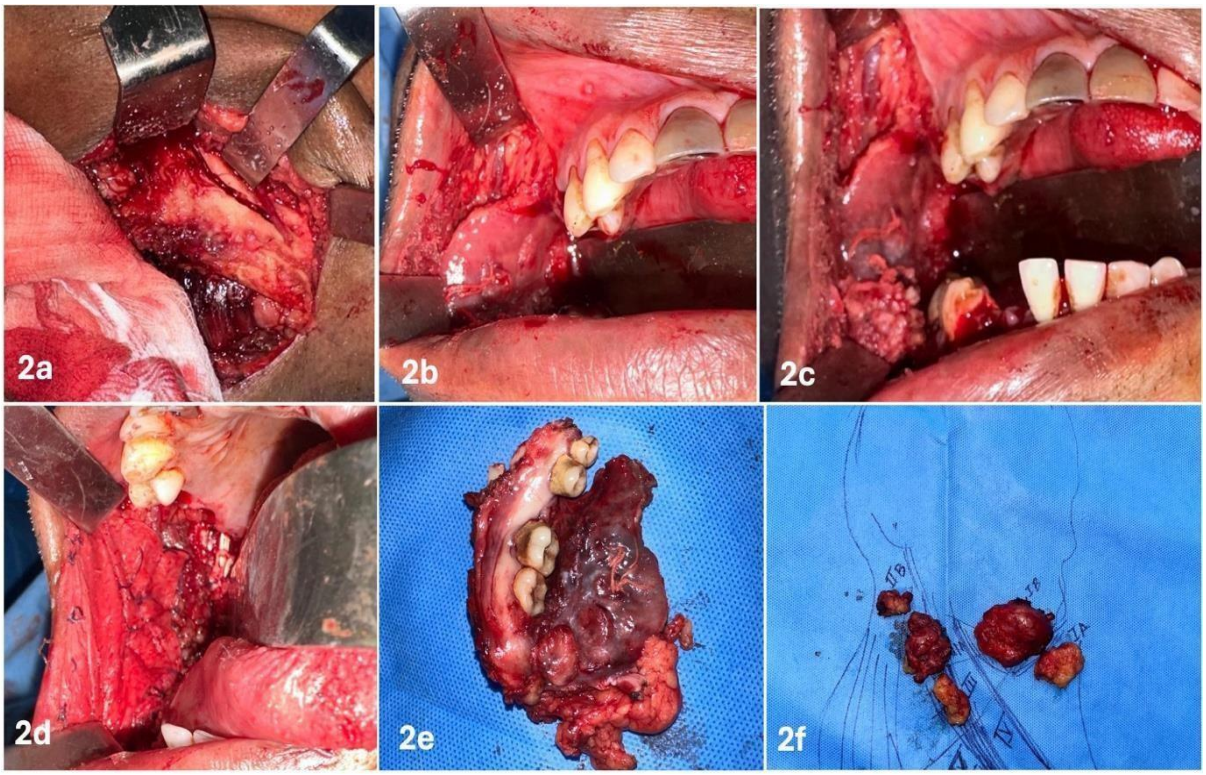


Figure 2. Intraoperative pictures showing 2a. Osteotomy cut for marginal mandibulectomy, 2b and 2c. Wide local incision of the lesion in the right buccal mucosa, 2d. Lesion excised and reconstructed with Myriad matrix, 2e. Excised specimen- buccal mucosal lesion along with marginal mandibulectomy, 2f. Excised specimen showing selective lymph node dissection (level I to III)



Figure 3. 3a. Postoperative image showing no gross deformity at the 13th month follow-up visit, 3b. Intra oral complete mucosalization following myriad application

Case description 2.

A 56-year-old female presented to the outpatient department at a private hospital at Chennai, India, with a primary complaint of irritation caused by a sharp lower right third molar (tooth 48), which was reportedly impinging on the adjacent right lateral border of her tongue. The patient gave a history of a non-healing ulcer over the right side of the tongue which had persisted for several weeks. She had undergone an incisional biopsy 15 days prior, which showed inflammatory cells but was negative for malignancy.

Clinical examination of the oral cavity revealed a non-healing ulcer measuring approximately 0.5 cm × 0.5 cm on the right lateral aspect of the tongue. The ulcer had raised and indurated margins and was supposedly in continuous contact with tooth 48 during speech and mastication. No cervical lymphadenopathy was detected on palpation. Due to the suspicious nature of the lesion, an excision biopsy was performed for a revision surgery to obtain free margin, along with extraction of tooth 48. Histopathological examination of the excised specimen suggested squamous epithelium with underlying neoplasm arranged in sheets and nests composed of large polygonal cells with nuclear pleomorphism, hyperchromasia, increased mitosis and moderate amount of eosinophilic cytoplasm. These features confirmed the diagnosis of moderately differentiated keratinizing squamous cell carcinoma of the tongue.

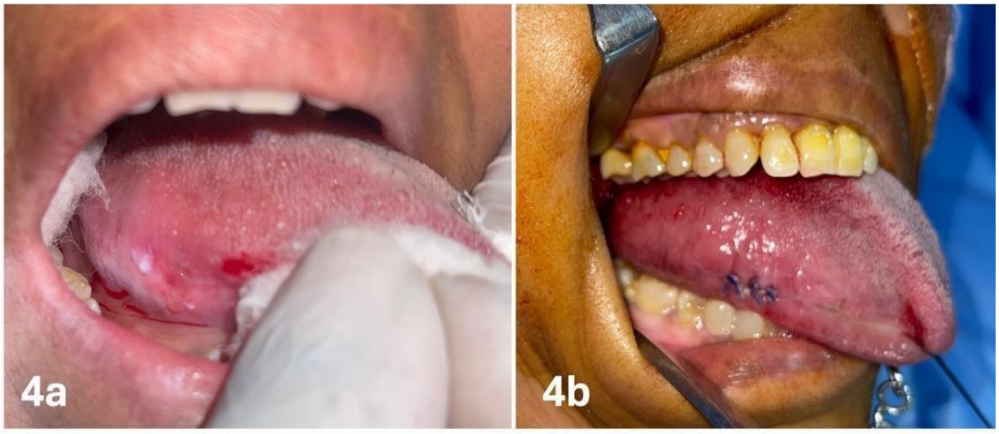


Figure 4. 4a. Intraoral image showing suspicious tender and indurated ulcerative lesion (0.5 cm x 0.5 cm) over the right tongue, 4b. Post-operative sutured wound following the excisional biopsy

Surgical management and post-operative follow-up

Intraoperatively, the lesion was excised with a 1 cm oncological margin, followed by selective neck dissection involving levels I to III. The tongue defect resulted in a partial glossectomy with minimal muscle involvement. Reconstruction of the defect was performed using a myriad (extracellular matrix) graft. Postoperative healing was satisfactory, with the patient exhibiting good tongue movements, speech and deglutition.





Figure 5. 5a. Surgical excision of the lesion, 5b. Excised tongue tissue along with evidence of clear margins, 5c. Myriad matrix soaked in saline before application, 5d. Myriad placed in situ & adapted well by quilting technique

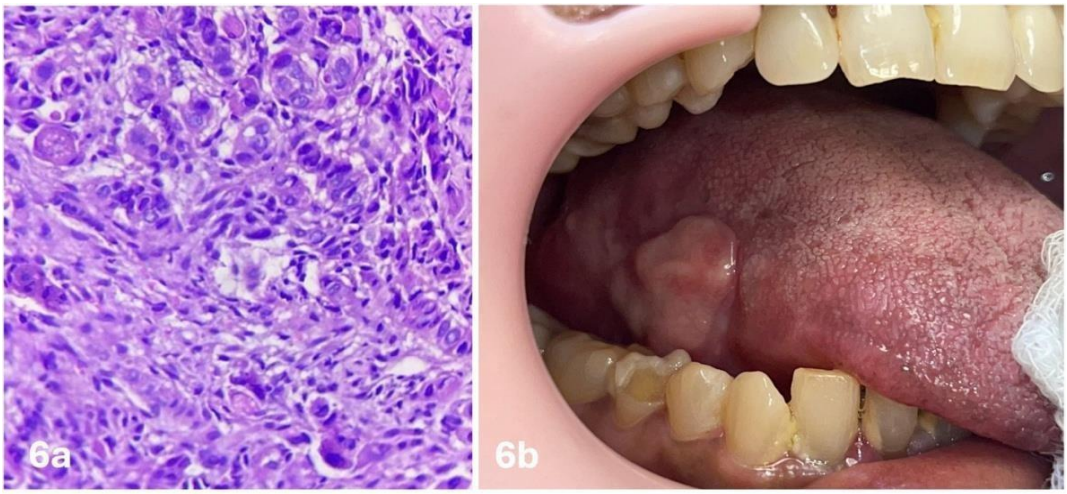


Figure 6. 6a. Histopathological image of the excised specimen suggestive of moderately differentiated keratinizing squamous cell carcinoma, 6b. 2 month post-operative image showing mucosalization of grafted tongue site

DISCUSSION

The management of oral squamous cell carcinoma pose significant challenge to clinicians as it not only involves oncologic resection, but also functional reconstruction of the resultant defect site [11]. In particular, the oral cavity being considered a sensitive site due to its constant mobility and microbial exposure, may not be amenable for an unhindered healing process, thus making the reconstruction of such sites quite demanding to garner long-term function outcomes.

In both the cases described here, the resection of the oral SCC resulted in a sizeable soft tissue defect. While the first case involved a wide excision of the right buccal mucosa, extending to the mandible, the second one warranted a partial glossectomy of the right aspect of the tongue. Each of the case exhibited unique challenges with respect to preserving aesthetics, form and function while minimizing morbidity and achieving superior stability.

In both the patients, the Myriad matrix (OFM) was grafted to the defect site following resection, circumventing the donor site and thus, multiple-step surgical procedures. In the first case with the wide mucosal defect and marginal mandibulectomy, the placement of the Myriad matrix allowed for tension free closure and acted as an ideal scaffold for re-epithelization and tissue integration. The intraoral closure was carried out using the 3-0 vicryl over the matrix and the extra oral closure via staples. The healing was satisfactory and the immediate, post-operative functional outcomes, comprising of speech, mastication, deglutition, and mobility, were adequately re-established. Although marginal mandibular defects are customarily reconstructed using free flaps, in the present case, the Myriad matrix was effectively employed to graft both the marginal mandibular defect and the associated buccal mucosal defect. In the second case, the defect site after partial glossectomy was reconstructed using a single, fine, OFM layer, trimmed and immobilized over the excised area. Post-operative evaluation revealed unexceptional healing, good tongu

movement, phonation, adequate articulation, with no evidence of complication or graft failure.

These findings of the present case series are supported by Su *et al.*, who described similar effective use of the Myriad matrix in tongue defect sites reconstruction. The matrix integration with tissue architecture occurred within three weeks' time with complete mucosal resurfacing and negligible scarring [10]. No adverse events were observed and the patients maintained stable function even with chemotherapy.

In a report by Consorti *et al.*, dermal matrix reconstruction of intraoral SCC resective procedures in 47 patients demonstrated favorable outcomes in buccal mucosal and tongue defects. While two cases of membrane failure were observed, all other patients demonstrated excellent mucosal healing and re-establishment of function at six months follow-up [12]. In addition, these findings echo our own experience with OFM in minimizing morbidity and maximizing functional recovery. Moreover, Bohn and Chaffin in 2020 reported that OFM reconstructed in soft tissue defects on denuded vital structures, showed defect granulation within 1-2 weeks and complete granulation tissue formation with 1-6 weeks [9].

Thus, it is evident that the Myriad matrix renders key advantages in the reconstruction of oral cavity defects, making it a potent alternative to conventional reconstructive biomaterials. Unlike many other biomaterials, it demonstrates tremendous resistance to enzymatic degradation in a saliva-rich environment, ensuring durability amidst the vital phases of healing [13]. Moreover, the matrix also refrained from the necessity of microvascular anastomosis, thus making the overall procedure relatively less technique sensitive.

It has also been suggested that the mucosal lining that regenerates over the matrix highly mimics native oral mucosa. Unlike the split-thickness grafts, that frequently result in keratinized or fibrotic tissue, OFM bolsters the restoration of a soft and pliable, non-keratinized mucosal phenotype, and thus, considered ideal for re-establishing speech, mastication, and deglutition. By and large, these advantages translate to a cut-above in the management of oral SCC patients.

CONCLUSION

The use of the Myriad matrix soft tissue bioscaffold or the OFM in the reconstruction of oral post-cancer excision defects exhibits a viable, efficient, and biologically amenable substitute to conventional reconstructive biomaterials. In our present cases, oral SCC of buccal mucosa and tongue, the OFM grafts culminated in exceptional healing, tissue regeneration, and preservation of oral functions and esthetics with no untoward complications. Thus OFM may be considered a frontline reconstructive option, especially for intraoral defects. With more studies and refinement, it holds promise to become a principal component in oral and maxillofacial reconstructive surgeries.

DECLARATIONS

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CASE REPORT

SURGICAL SUCCESS OF OVINE FORESTOMACH MATRIX IN THE MANAGEMENT OF PLEOMORPHIC ADENOMA OF HARD PALATE -CASE REPORT

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ABSTRACT

Pleomorphic adenoma is one of the common benign salivary gland tumors, occurring in the hard palate due to a high density of minor salivary glands in this region. Surgical excision remains the mainstay of management; however, defect site reconstruction following tumor excision poses a considerable challenge. The current case report presents the first known use of the ovine forestomach matrix (OFM) for reconstructing the huge defect post excision. A 40-year-old patient male presented with a progressively enlarging, painless swelling in the hard palate. Clinical examination revealed a well-demarcated, fluctuant, non-tender lesion, crossing the midline, with a provisional diagnosis of mucocele. Surgical excision was performed with wide margins to prevent risk of recurrence. The OFM was employed as a biologic scaffold to facilitate wound healing and tissue regeneration. The patient demonstrated exceptional healing with full mucosal coverage and no signs of infection or necrosis at the nine-month follow-up. The successful integration of OFM in this case underscores its potential as a novel alternative adjuvant for reconstructing oral and maxillofacial defects. This case sets a precedent encouraging further research to elucidate its long-term efficacy and expand its application in maxillofacial surgical interventions.

Keywords: **Pleomorphic Adenoma, Salivary Gland Neoplasms, Hard Palate, Wound Healing, Ovine Forestomach Matrix, Biocompatible Materials**

INTRODUCTION

Pleomorphic adenoma is one of the most commonly occurring benign tumours of the salivary glands, primarily attributed to the parotid gland, but can also occur in the minor salivary glands. Previous reports in the literature account this condition for about 40 to 70% of all the major and minor salivary gland tumours [1].

It is also designated as the 'mixed tumour' owing to its composition, encompassing the epithelial and the mesenchymal elements [2].

The condition is characterized by a gradually growing, painless, submucosal firm mass, occurring in different parts of the oral cavity, including the hard palate, the upper lip, the floor of the mouth and the buccal mucosa. Clinically, the growth is encapsulated and well demarcated, though in some situations, it may infiltrate into the surrounding tissues, in which case it is more likely to be prone for recurrence [3]. It presents with a smooth or lobulated surface, generally, not ulcerated, unless subjected to trauma. The exact etiology of pleomorphic adenoma is still unclear; however, it is believed to arise from the intercalated ductal and myoepithelial cells of the salivary glands. In addition, genetic variations, such as the chromosomal aberrations involving PLAG1 and HMGA2 genes, have been reported to play a role in the pathogenesis of this condition [4, 5].

Pleomorphic adenomas shows a predilection towards adults, slightly towards females with highest occurrence between the third and the fifth decades of life. It frequently occurs in the hard palate accounting for over 50% of the cases, probably due to the high density of minor salivary glands in this particular region. Despite being a benign lesion, a small percentage of cases may show malignant transformation, particularly in long-standing or recurrent conditions. Literature reports varied prevalence, with up to 70% accounting to minor salivary gland neoplasm. This benign condition may cause functional derangement such as difficulty in speech, mastication, and swallowing, eventually affecting the patient's quality of life. The principal treatment modality for pleomorphic adenoma is complete surgical excision of the lesion and careful histopathological evaluation to rule out malignant transformation [6]. In cases of bone invasion or subjacent structures, extensive surgical approaches including maxillectomy or the usage of several matrices for grafting the huge defect may be warranted.

The ovine forestomach matrix (OFM) is known for its excellent regenerative potential. It has proven to promote cell migration, angiogenesis and tissue remodelling, thereby making it highly useful for supporting huge soft tissue defects [7]. Reports have suggested that OFM inhibits the release of inflammatory mediators like matrix metalloproteinase and neutrophil elastase, thereby reducing the exaggerated inflammatory status in and around the defect site, further preventing excessive tissue degradation [8]. In addition, the OFM has also proven highly beneficial in chronic wound management. In this case, the OFM was used in the surgical management of the palatal defect following pleomorphic adenoma excision. In addition, as the recurrence is reported to be 5-50%, proper and meticulous post-operative follow-up is key to monitor complete resolution, prevent recurrence and further complications.

Here, we present a case of utilising OFM which played a critical role in the surgical management of pleomorphic adenoma of the hard palate in a male patient as it provided structural integrity to the defect site, facilitated wound healing and presented no complications. The outstanding post-operative outcome of OFM usage in this case highlights its potential as a valuable adjuvant in oral and maxillofacial surgical management.

Case description

History

A 40-year-old male patient presented with a complaint of swelling in the roof of the mouth for the past six months and was thus referred to the department of Oral and Maxillofacial Surgery at Sri Ramachandra Dental College for evaluation and management. The patient noticed a small swelling on the hard palate, which gradually increased to the current size. History suggested that the swelling was not associated with pain or discharge. The patient had no contributory past medical or surgical history. His personal history was unremarkable, with normal bowel and bladder habits, as well as adequate sleep and appetite. He also showed no history of deleterious habits.

Clinical Examination and Treatment Plan

On general examination, the patient was conscious, coherent, and afebrile. The vital signs were within normal range. Extraoral examination revealed no gross facial asymmetry, and no abnormalities were detected in the temporomandibular joint. The patient had adequate mouth opening and normal lateral jaw movements. No palpable lymph nodes were detected.

On intraoral examination, a swelling measuring approximately 5 cm in diameter on the hard palate was apparent, which extended from the anterior hard palate to the hard and soft palate junction, further reaching the region of the tooth, 27 and also crossing the midline. The lesion presented with a smooth and glistening surface with secondary erythematous

changes. On palpation, the swelling was soft, fluctuant, non-compressible, non-reducible, and non-tender. There was no evidence of discharge. The tooth 26 was tender on percussion, and a root stump was present in relation to 46 region. Generalized stains and calculus were also observed. Occlusion was stable. Computed Tomography revealed mild rarefaction in the lateral aspect of the palatal bone. Intraoral occlusal radiograph and orthopantomogram revealed no evidence of bony invasion (Figures 1, 2 and 3). Based on clinical findings and parameters, a provisional diagnosis of a mucocele in relation to the hard palate was arrived. Differential diagnoses included osteoid osteoma, fibrous dysplasia, maxillary tori, adenoid cystic carcinoma, and pleomorphic adenoma. These lesions were considered for differential diagnoses owing to their clinical presentation and similarity to the observed swelling in the hard palate. The treatment plan included surgical excision of the lesion under general anesthesia.

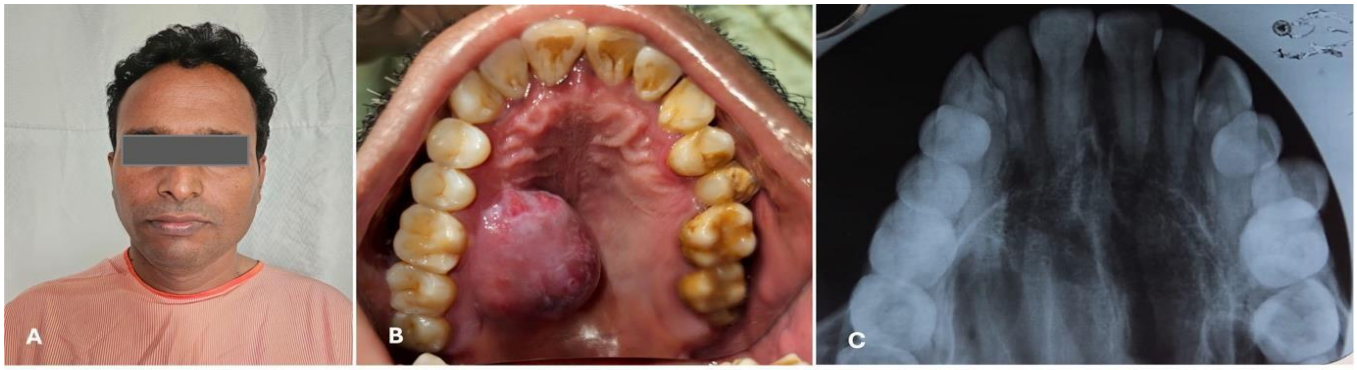


Figure 1. Shows the extraoral and intraoral findings (A and B); corresponding radiograph (C)



Figure 2. Shows the orthopantomogram with no evidence of bony invasion

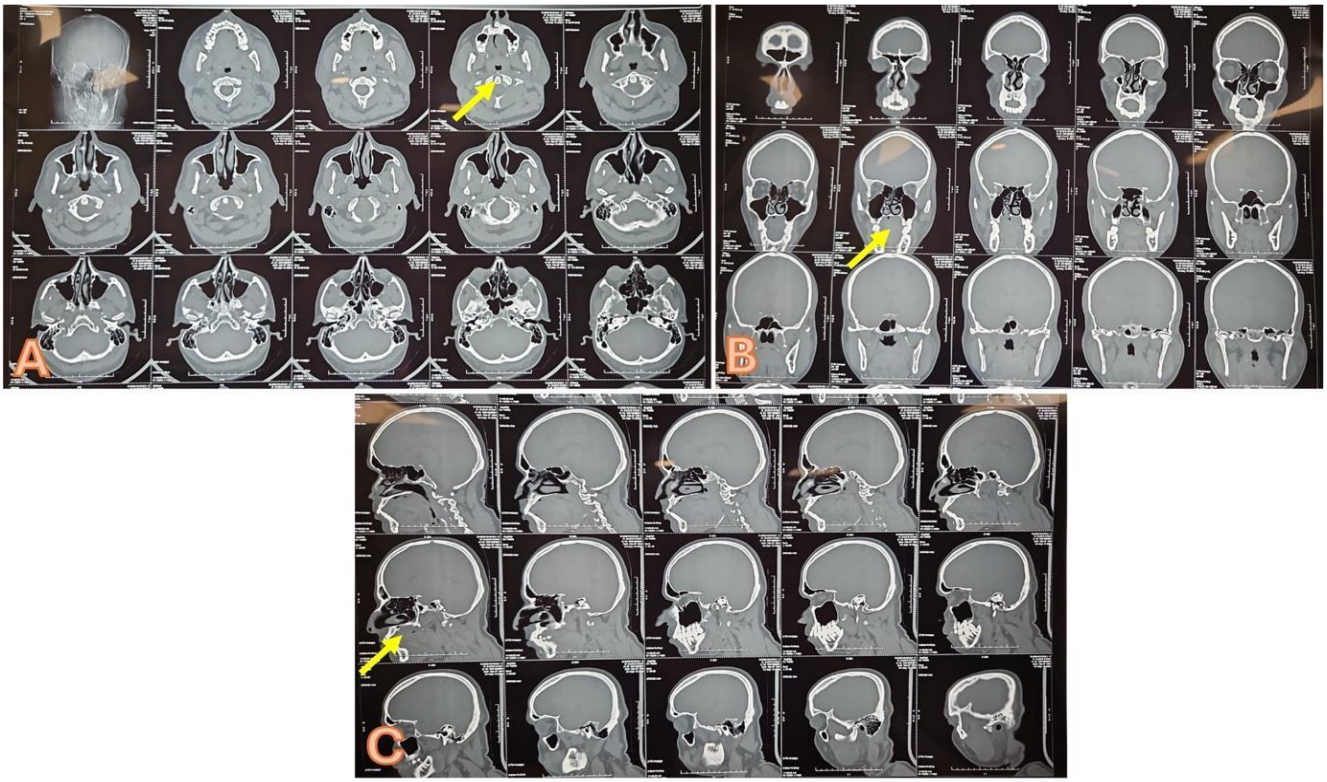


Figure 3. CT showing mild rarefaction (highlighted by arrows)

Surgical management

The patient underwent surgical excision of the lesion under general anesthesia. He was placed in a supine position, and general anesthesia was administered with nasal intubation. The surgical site was prepared with intraoral and extraoral antiseptic application using 10% povidone-iodine, and the patient was draped under sterile conditions. Fine needle aspiration was performed, followed by an elliptical incision around the lesion. Subperiosteal dissection was carried out to separate the mass from the underlying hard palate, and the lesion was excised in toto. Saline and betadine wash were given, and hemostasis was achieved. Sharp bony margins were smoothened, and the surgical site was packed with OFM (Endofoam^{TM}, Natural Dermal Template). The site was sutured using 2-0 and 3-0 Vicryl sutures. Bactigras dressing was applied, and an acrylic palatal plate was placed and secured with wire (Figures 4 and 5).

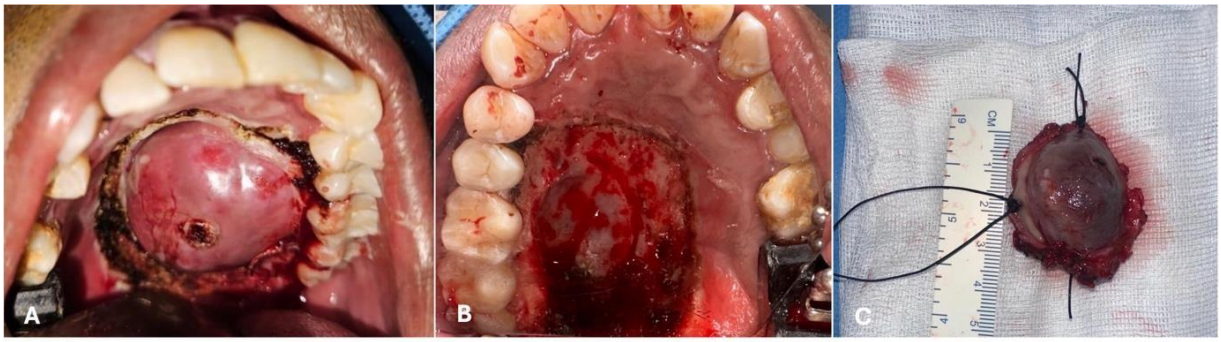


Figure 4. Intra-operative images showing planned margins of incision (A); post excision (B); Excised specimen to be sent for histopathological evaluation (C)

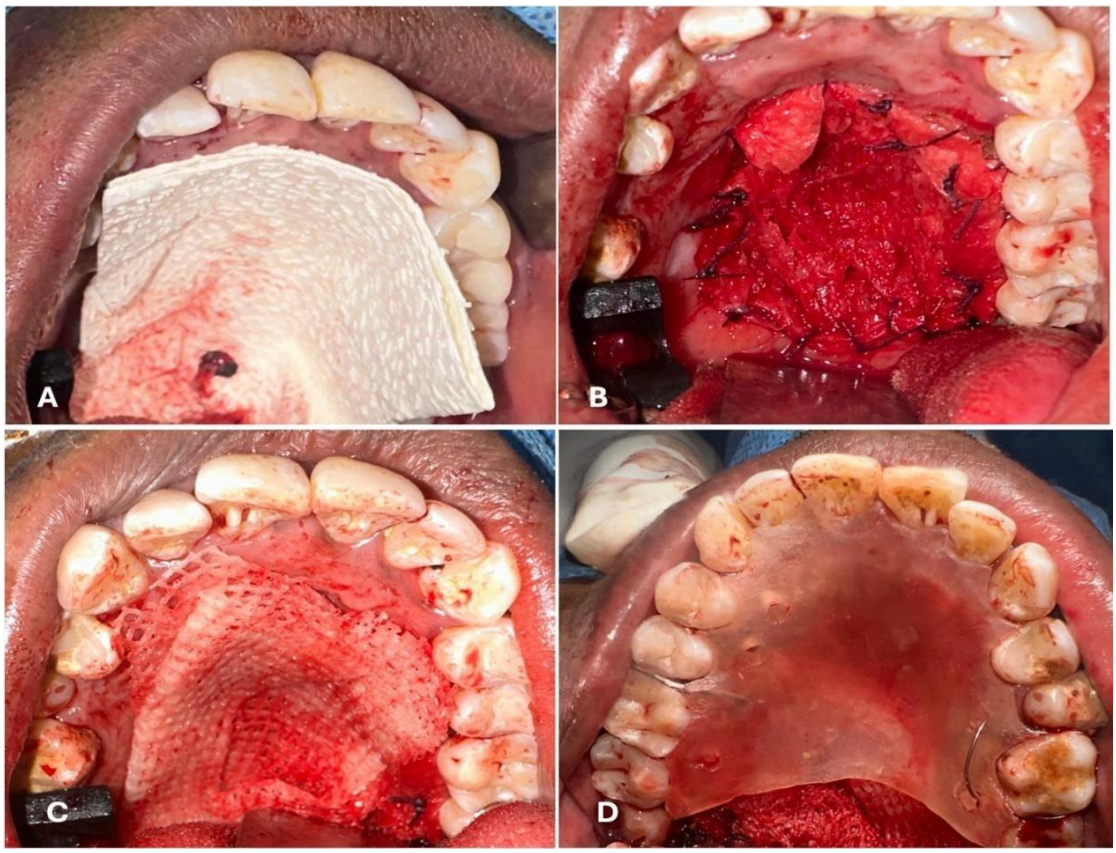


Figure 5. Intra-operative images showing OFM adaptation (A); OFM, post suturing (B); Bactigras placement (C); Palatal plate covering the post-operative site

Histopathological features

The histological section revealed cuboidal cells arranged in a ductular pattern, containing eosinophilic coagulum. Additionally, spindle-shaped cells were organized in strands with areas of hyalinization. There were also regions of squamous differentiation forming keratin pearls, along with chondromyxoid areas. The overlying palatal mucosa and mucus acini were also observed. All the features were suggestive of pleomorphic adenoma.

Post-Operative care

Following the procedure, the patient was extubated in a stable condition and shifted to the post-anesthesia care unit (PACU) for further observation. Postoperative care included pain management, maintenance of oral hygiene, and instructions for follow-up to monitor healing and prevent complications.

At 9-month follow-up

The nine-month post-operative follow-up demonstrated the defect site with excellent healing and complete mucosal coverage over the surgical site. The palatal tissue appeared well-integrated, with no indication of ulceration, dehiscence or necrosis. The colour and texture of the palatal mucosa blended seamlessly with the surrounding tissues. These clinical findings indicated complete epithelialization and successful integration of the ovine forestomach matrix. In addition, there was also no evidence of infection, fibrosis, or scar tissue formation which, all together, suggested optimal wound healing and restoration of palatal tissue integrity. These findings reinforce the effectiveness of the OFM in facilitating post-surgical defect closure and tissue regeneration (Figure 6).



Figure 6. Postoperative view at 2 weeks follow-up (A); 9 month follow-up (B)

DISCUSSION

Pleomorphic adenoma of the hard palate is a rare yet an important clinical entity that warrants prompt diagnosis and appropriate surgical intervention [9]. Any undue delay may only lead to complications, including local bony invasion, and malignant transformation. The present case highlights the surgical excision of a palatal pleomorphic adenoma and the novel application of ovine forestomach matrix (OFM) in defect reconstruction, demonstrating exceptional post-operative healing. To the best of our knowledge, this is the first reported case in literature, where OFM has been employed in managing a palatal lesion, underscoring its potential in oral and maxillofacial surgery.

Studies have indicated that prolonged presence of pleomorphic adenoma can result in osseous invasion, recurrence, and in some cases, even malignant transformation into carcinoma. Since the lesion customarily presents as slow-growing and painless, patients often tend to neglect the condition, allowing it to enlarge and plausibly infiltrate surrounding structures. Lesion infiltration can further complicate surgical intervention, upsurging the necessity for aggressive modalities, such as maxillectomy, leading to significant morbidity. The pseudo capsule of the lesion abodes microscopic extensions of neoplastic cells, contributing to recurrence in incomplete excisions [10]. This highlights the significance of wide surgical excision with clear margins to prevent remnants and recurrence.

Several biomaterials have been explored as adjuncts for wound healing and defect reconstruction following wide tumor excision [11, 12]. Conventionally, acellular dermal matrices, xenografts, collagen-based matrices, etc., have been utilized to support soft tissue regeneration. Ovine forestomach matrix (OFM) is a relatively novel biologic matrix that has obtained attention owing to its superior regenerative potential.

Unlike traditional matrices, OFM has demonstrated it property to retain its native extracellular components, including glycosaminoglycans and proteoglycans, which are pivotal for tissue remodeling and angiogenesis. Although, the matrix has been extensively applied in chronic wound management, hernia repair, and lower-extremity soft tissue defects, its application in oral and maxillofacial surgery, remains elusive [13]. Apart from matrices, other adjuvant approaches have been incorporated while managing PA excision such as negative pressure wound therapy, and platelet-rich fibrin (PRF). However, these modalities may not always prove sufficient in wider defects where structural integrity must be maintained, warranting the use of biomatrices such as OFM.

In this case, OFM acted as a biocompatible scaffold facilitating, cellular infiltration, neovascularization, and tissue integration [14]. In addition, it also helped prevent excessive tissue degradation and ensuring a stable wound environment. The excellent wound healing noted in this case could be attributed to the ability OFM to integrate well with both soft and hard tissues. The matrix was placed in direct contact with the bony surface of the palate, and its porous structure would have facilitated conducive grounds for guided tissue regeneration, leading to excellent post-operative healing [7].

The success of OFM in this case suggests its potential as a viable alternative for defect reconstruction in oral and maxillofacial surgery. Future studies are needed to demonstrate its long-term outcomes in oral surgical procedures, will serve as a benchmark for its expanded applications beyond the traditional use in soft tissue repair.

CONCLUSION

Surgical excision remains the mainstay in the management of pleomorphic adenoma and the choice of reconstructive biologic matrix plays a pivotal role in post-operative outcomes. The use of OFM in this case proved exceptional in terms of wound healing,

demonstrating its potential in oral and maxillofacial surgical interventions. The case also underscores its clinical efficacy and versatility for being considered a viable option for maxillofacial applications.

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Conflict of interest

The authors declare no conflict of interest

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Ethical Approval

"Not applicable"

Consent for publication

"Appropriate consent obtained"

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RESEARCH ARTICLE

EFFICACY OF LAETRILE IN TREATMENT OF CANCER PATIENTS WITH CHEMORADIATION ORAL MUCOSITIS

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ABSTRACT

Background: Post-chemoradiation mucositis is a common and serious side effect that occurs in patients undergoing cancer treatment. It is an inflammatory condition of the mucous membrane that can cause significant discomfort, pain, and a significant reduction in quality of life. Among the many methods used to relieve and treat mucositis, the drug Laetrile has attracted particular attention due to its potential therapeutic properties.

The aim of this study was to evaluate the effectiveness of Laetrile in treating post-chemoradiation mucositis.

Materials and Methods;The study involved 89 patients diagnosed with cancer of the oral mucosa (C00-C06, C10, C13, C14 according to ICD-10) stages II and III (T2-3, N0-1, M0), aged 55 to 67 years, who received chemoradiation therapy to the head and neck area. Depending on the treatment method, patients were divided into two groups: comparison traditional treatment) and the main (developed method that reduced the incidence of bleeding gums, erosions and ulcers of the oral mucosa.

Results: The use of Laetrile allowed to achieve a therapeutic-prophylactic effect in 55 (93.2%) of 89 patients treated with Laetrile while in the comparison group (traditional treatment) in 19 (63.3%) of 30 patients

Conclusion: Laetrile is an interesting alternative in the treatment of postchemoradiation mucositis thanks to its potential ability to reduce inflammation and promote tissue regeneration. Thus, although Laetrile is a promising option for managing the symptoms of mucositis, its potential benefits and risks have yet to be clarified in further research.

Keywords: oral mucositis, treatment, rehabilitation

INTRODUCTION

Head and neck tumors represent a significant group of malignant neoplasms, characterized by a gradual increase in incidence[1]. Despite the progress in the field of early diagnosis and treatment of oncological diseases, complex antitumor treatment using chemotherapy and radiotherapy is inevitably accompanied by changes in the surrounding normal tissues (destructive, erosive-ulcerative and necrotic changes[2-4].

Currently, chemotherapy remains one of the most common treatment methods, despite the progress made in recent years in the management of oral and maxillofacial (OMF) cancer[5-7].

It is used either alone or in combination with other therapeutic methods, preventing the proliferation of atypical cancer cells. The oral cavity is very vulnerable to the direct and indirect toxic effects of chemotherapy, which is due to the lack of selectivity of the targeted cells, as a result of which both tumor cells and normal cells are damaged. This is due to the specific features of the oral cavity, which include a high level of turnover of mucosal cells, the presence of a complex and diverse microflora in the oral cavity, as well as minor traumatic injuries resulting from the implementation of normal oral functions (chewing, swallowing, vocalization).

Mucositis is one of the most common side effects of antitumor therapy, significantly reducing the quality of life of cancer patients[8-10].

Oral mucositis generally begins 5-10 days following the initiation of chemotherapy and duration depends on the type, dose, and course of treatment[11,12].

On average, the incidence of mucositis is 29–66% the most severe complications develop in patients receiving high-dose radiation therapy for head and neck cancer[13].

The development of mucositis leads to interruptions in courses of antitumor treatment and, as a consequence, a decrease in its effectiveness, which negatively affects the prognosis of the disease[13-15].

Pathogenesis of radiation-chemotherapy mucositis the following five-stage model [16] , is based on the evidence available to date (figure 1).

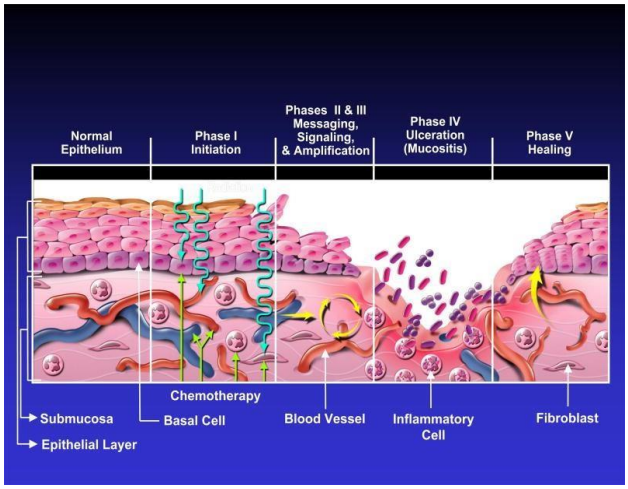


Figure 1. Current five-phase pathobiologic model of oral mucositis. (Reprinted from Sonis ST. A Biological Approach to Mucositis. J Support Oncol 2004; 2:21–36).

The degree and extent of oral mucositis that develops in any particular patient and site appears to depend on factors such as age, gender, underlying systemic disease and race as well as tissue specific factors (e.g. epithelial types, local microbial environment and function)[17].

A wide variety of scales have been used to record the extent and severity of oral mucositis in clinical practice and research. The World Health Organization (WHO) scale combines both subjective and objective measures of oral mucositis.

Mucositis is graded using the World Health Organization (WHO) Mucosal Injury Scale[17](Table 1).

Table 1.

World Health Organization (WHO) scale for oral mucositis

Grade 0 = No oral mucositis
Grade 1 = Erythema and Soreness
Grade 2 = Ulcers, able to eat solids
Grade 3 = Ulcers, requires liquid diet (due to mucositis)
Grade 4 = Ulcers, alimentation not possible (due to mucositis)

The National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 includes separate subjective and objective scales for mucositis [18].

National Cancer Institute (NCI) Oral Mucosal Injury Scale (Table 2).

National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 3.0

Oral mucositis (clinical exam)
Grade 1 = Erythema of the mucosa
Grade 2 = Patchy ulcerations or pseudomembranes
Grade 3 = Confluent ulcerations or pseudomembranes; bleeding with minor trauma
Grade 4 = Tissue necrosis; significant spontaneous bleeding; life-threatening consequences
Grade 5 = Death
Oral mucositis (functional/symptomatic)
Grade 1 = Minimal symptoms, normal diet
Grade 2 = Symptomatic but can eat and swallow modified diet
Grade 3 = Symptomatic and unable to adequately aliment or hydrate orally
Grade 4 = Symptoms associated with life-threatening consequences
Grade 5 = Death

The WHO Mucosal Injury Scale is the most commonly used scale in clinical and research settings, while the NCI CTCAE scale is often used as a measure of overall toxicity.

Signs and symptoms of mucositis include:

-Red, shiny, or swollen mouth and gums;

-Blood in the mouth;

-Sores in the mouth or on the gums or tongue;

-Soreness or pain in the mouth or throat;

-Difficulty swallowing or talking;

-Feeling of dryness, mild burning, or pain when eating food;

-Soft, whitish patches or pus in the mouth or on the tongue;

-Increased mucus or thicker saliva in the mouth.

Oral mucositis can:

-Cause pain;

-Restrict oral intake;

-Act as a portal of entry for organisms;

-Contribute to interruption of therapy;

-Increase the use of antibiotics and narcotics.

In addition, mucositis significantly increases the cost of treating cancer patients and increases the duration of hospitalization. In this regard, there is a need to search for and develop.

The main clinical signs of mucositis include: oral pain of varying intensity, dry mouth, inability to eat. To reduce the severity of mucositis caused by chemo- and / or radiation therapy, it is proposed to develop multidisciplinary guidelines for oral care, familiarize staff and patients with them.

Oral Care: Standardized oral care protocols are recommended to prevent oral mucositis in all age groups and across all cancer treatment modalities (LOE III).
Mucositis Study Group of the Multinational Association for Supportive Care in Cancer and the International Society of Oral Oncology (MASCC/ISOO) has developed clinical practice guidelines for the management of mucositis[19-21]. Management of oral mucositis is divided into the following sections: nutritional support, pain control, oral decontamination, palliation of dry mouth, management of oral bleeding and therapeutic interventions for oral mucositis.

Good oral care regimen can help prevent or decrease the severity of mucositis and, just as important, help prevent the development of infection through open mouth sores. The mainstay of an effective oral care regimen is mouth rinses,Such recommendations should include mechanical cleaning (brushing teeth with a soft brush, dental floss), rinsing the mouth to reduce the accumulation of bacteria (soft rinses), and moisturizing and lubrication (applying moisturizers) to the surface of the oral mucosa. In the presence of minor pain in the mouth, treatment should begin with rinsing. If they are insufficiently effective, local anesthetics are added (for example, 2% lidocaine solution). In the presence of extensive painful ulcers, systemic analgesics are added to the treatment.

Currently, various drugs are recommended for the treatment of inflammation of the mucous membranes, including herbal ones[22-23].

Use of natural medicinal herbs for the treatment of complications and side effects Cancer therapy gained strength in the last few decades and became a new area researches. In the context of symptoms in the treatment of radio-induced mucositis, the effectiveness of chamomile, aloe vera, honey extract is currently being studied, Manuka, vitamin A, glutamine[25-26].

Among the variety of methods used to relieve and treat mucositis, the Laetrile attracts special attention due to its potential therapeutic properties[27].

Laetrile is a cyanogenic glycoside, also known as vitamin B17, is a derivative of amygdalin, a compound found in the seeds of fruits such as apricots, peaches, and almonds. Laetrile has been used in the past as an alternative cancer treatment, but its effectiveness in the fight against cancer remains controversial[28]. However, interest in its use in the treatment of post-chemoradiation mucositis has increased, as the compound may have the ability to reduce inflammation and stimulate tissue regeneration..In the 1950s, an intravenous form of amygdalin was patented and named laetrile, a semisynthetic compound composed of D-glucuronic acid and mandelonitrile.

One possible mechanism of action of Laetrile is its ability to stimulate apoptosis of damaged cells, which may speed up the healing process of the mucosa[29-31]. In addition, some studies point to its role in modulating the immune response and reducing inflammation, which may also be useful in mucositis[32,33].

The aim of this study was to evaluate the effectiveness of Laetrile in treating post-chemoradiation mucositis.

MATERIAL AND METHODS

The clinical study was conducted at the State Budgetary Healthcare Institution of the Republic of Crimea"Crimean Republican Oncology Clinical Dispensary named after V. M. Efetov". The study included 89 patients diagnosed with cancer of the

cervical spine (C00-C06, C10, C13, C14 according to ICD-10) stages II and III (T2-3, N0-1, M0), aged 55 to 67 years (the average age was 63.6±4.35 years), of which 69 were men (77.53%), 20 were women (22.47%), who received chemoradiation therapy to the head and neck area. Antitumor treatment was performed by an oncologist according to clinical recommendations according to the scheme: single focal dose (SFD) from 1.8 to 2 Gy, total focal dose (TFD) from 40 to 60 Gy using the gamma installation of the linear apparatus Elekta Compact TM, Versa HD [2]. Chemotherapy with cisplatin at 100 mg/m2 intravenously at a rate of no more than 1 mg/min with pre- and posthydration every 3 weeks (on the 1st, 22nd and 43rd days during RT) and carboplatin at 1.5-2.0 AUC (during RT).

During the initial examination of the patient, complaints were clarified in a conversation, and a detailed anamnesis of the disease and life was collected. In this case, much attention was paid to identifying premorbid factors as predictors of the disease, namely: the presence and duration of bad habits, occupational hazards, a burdened family history, comorbid pathology.

During the initial examination of the skin of the face and neck, special attention was paid to changes in color and contours. Examination of the organs and tissues of the oral cavity was carried out according to WHO recommendations (1997), determining the condition of the dentition, periodontium, the presence and volume of dental plaque. Of particular interest was the study of the oral mucosa: color, relief, moisture, hyperemia, swelling, the presence of elements of damage - papules, erosions, aphthae, ulcers, scars, etc. Mucositis was diagnosed according to the WHO classification (1979). The study included patients only with grades III (erythema and ulcers, inability to swallow solid food) and IV (ulcers and food intake impossible) mucositis.

Depending on the treatment method, patients were divided into two groups: the main group (59 patients: 44 men, 15 women) and the comparison group (30 people: 25 men, 5 women). In the main group, oral cavity sanitation was performed - professional oral hygiene, tooth extraction and filling of carious cavities, the use of soft toothbrushes and Mexidol-phyto toothpaste, which restores microcirculation, activates local immunity, has an anti-edematous effect, reduces bleeding, stimulates wound healing, including purulent ones, has an anti-inflammatory, metabolic, immunocorrective effect. Antiseptic treatment was performed with a 0.005% chlorhexidine solution and rinsing with Laetrile (15 drops per glass (200 ml) of water) 2 times a day for 2 weeks. The oral mucosa was anesthetized in the presence of ulcers and erosions before or after chemoradiotherapy by applying Desensetinan gel for 5-7 minutes, then washing it off with water; a collagen patch Suprasorb (natural collagen dressing (4X6X0.8 cm, 5 pieces) moistened with a solution (drops) of Laetrile B-17, which regulates the functioning of the cell membrane, removes oxidation products from the body, has an antiseptic and analgesic effect, was applied to the affected areas of the oral mucosa. The patients in the comparison group received traditional treatment - antiseptic treatment with 0.06% chlorhexidine solution and olive oil applications. Observation periods: before, on the 3rd, 5th and 7th days of observation.

RESULTS

During the initial visit, the main complaints of patients were general weakness, headache, increased nervous excitability, sore throat, thickening of saliva, severe dry mouth (hyposalivation), pain when eating and bleeding gums, tooth mobility and exposed necks, inability to eat solid food, loss of shine and redness of the oral mucosa and lips, burning and tingling sensations, plaque on the teeth and oral mucosa, the presence of "ulcers" that are painful to palpation, taste disturbances, and bad breath. Most of the examined patients were diagnosed with oral mucositis of the third degree of severity: with MN stage II - in 17.97% of cases, with MN stage III - in 48.0%. Mucositis of the fourth degree, respectively, in 12.35% and 21.34% of cases. Early manifestations of mucositis and objective symptoms in patients were characterized by hyperemia and swelling of the oral mucosa, bleeding gums, the presence of erosions covered with fibrinous plaque, painful on palpation, focal or confluent epitheliitis, erosive and ulcerative-necrotic processes in 72-84% of patients.

Most often (in 82-100% of cases), continued development of mucosal edema, isolated small-focal erosions, less often - confluent large-focal erosions, and bleeding gums were observed. In 30-55% of patients, focal or confluent epitheliitis, erosions and ulcers were detected. The absence of filiform papillae on the dorsal surface of the tongue ("varnished tongue") was detected. In 8 (8.9%) patients, mucositis was complicated by pseudomembranous candidiasis: a characteristic abundant cheesy coating on the tongue was determined.

Hemorrhagic manifestations in the main group appeared 2-3 times less often than in the control group. At the same time, erosions, ulcers, necrosis and confluent epitheliitis did not occur in any of the patients treated with Laetrile. During chemotherapy, 92% of patients, when describing saliva, called it “tasteless”,

"thick", tried to swallow, but did not swallow because of its "viscousness", spit or kept their mouths slightly open so that saliva flowed onto a napkin or into a container, for which they were forced to lie face down on the bed. 8% of patients did not complain.

The dynamics of rheological parameters of the oral fluid of patients in both groups during treatment are presented in Table 3.

Table 3. Dynamics of rheological parameters of oral fluid of patients in the main group and comparison group.

Indicator	Main group (n=59)		Comparison group (n=30)	
	Before treatment	After treatment	Before treatment	After treatment
Volume of oral fluid (ml)	0,14±0,04	0,43±0,14*	0,15±0,05	0,17±0,07
Rate of salivation (ml/min)	0,4±0,11	1,3±0,51*	0,4±0,10	0,5±0,12
Viscosity of oral fluid (relative units)	7,4±1,90	2,5±1,03*	6,8±1,98	6,3±2,33

*Note: * - reliability of differences in the parameters (p<0.05) in the main group compared to the comparison group.*

The salivation rate parameters in individuals receiving Laetrile tended to increase statistically significantly, in some cases approaching similar parameters in healthy patients. When using complex therapy with Laetrile, the salivation rate was significantly higher compared to the parameters obtained during chemoradiation therapy in the comparison group (with classical (traditional) therapy), while the salivation rate parameters in episodes complicated and not complicated by mucositis did not differ statistically.

The salivation rate in patients in the comparison group during treatment is shown in Fig. 2, and in the main group - in Fig. 3.

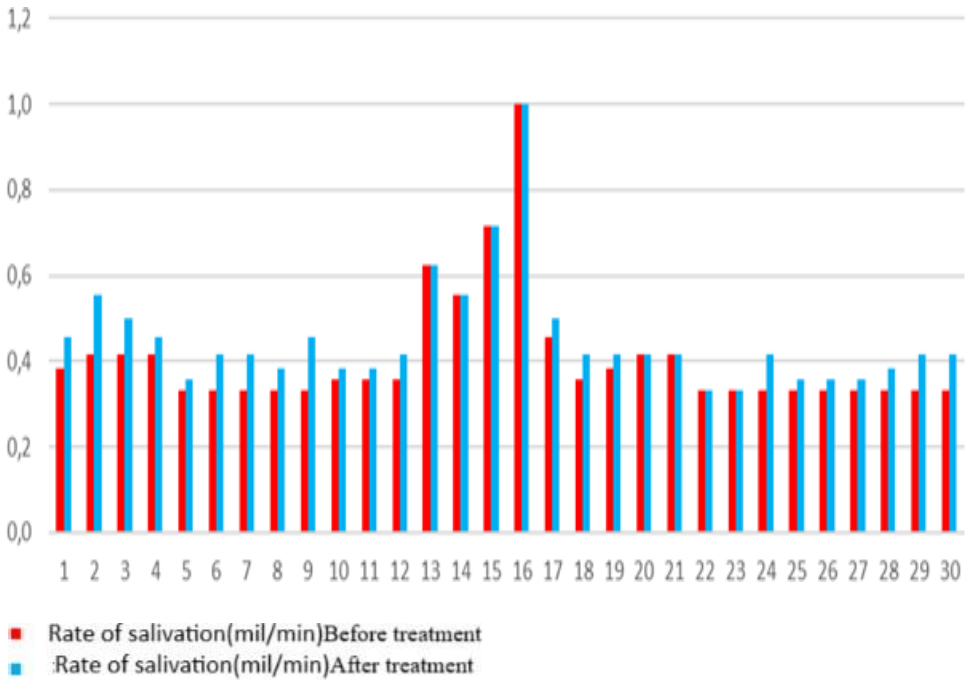


Figure 2. Rate of salivation in patients **(n=30)** of the comparison group during treatment

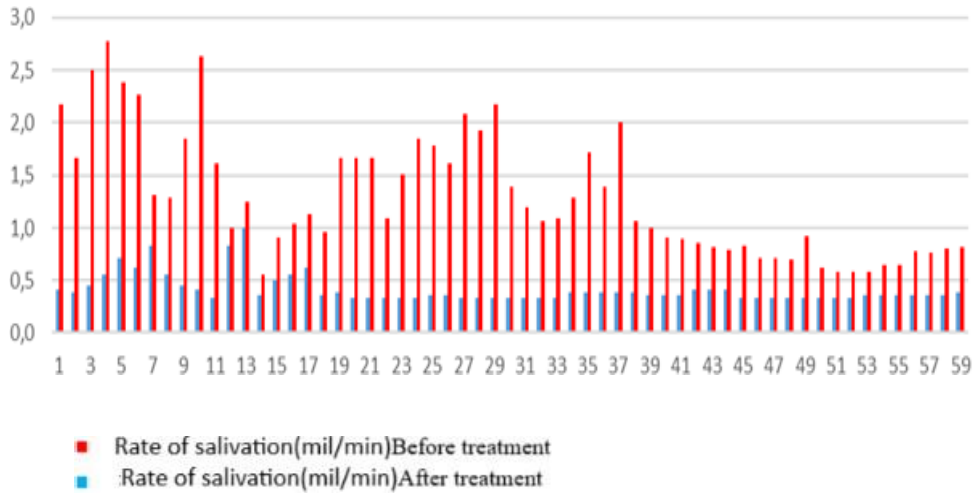


Figure 3. Rate of salivation in patients **(n=59)** of the main group during treatment

At the second stage of the study, the administration of Laetrile increased the volume of oral fluid in patients in the main group to 0.43±0.14 ml compared to the comparison group with standard therapy, where this figure was 0.17±0.07 ml ($p < 0.05$). There were no complaints from patients about the side effects of Laetrile during the use of Laetrile. From the data presented in Table 5.1, it is evident that when taking Laetrile, the individual indicators of saliva viscosity of patients were significantly lower (6.3±2.33 and 2.5±1.03), and the indicators of the rate of salivation were higher than those in the comparison group (1.3±0.51 and 0.5±0.12). Improvement of salivation characteristics had a positive effect on the well-being and behavior of cancer patients, since the quality and quantity of saliva did not interfere with its automatic swallowing, which confirms that the administration of Laetrile significantly improves the quality of life of cancer patients during chemotherapy and radiation therapy. Saliva viscosity in the comparison group significantly exceeds this indicator in the main group (according to median indicators, more than twice: 6.1 and 2.3, respectively; $p < 0.05$).

Figure 4 and figure 5 show the data on the viscosity of oral fluid in patients of the main group during treatment and the comparison group.

We provide diagrams with individual data for all patients to demonstrate the level of variability of their personal indicators of oral fluid rheology. With statistical significance of these parameters, there are cases that go beyond the average variation series, which is apparently associated with both the morphofunctional features of the salivary glands (primarily the parotid gland) and the features of the autonomic regulation of salivation. This is especially demonstrative in the comparison group and may be important for the doctor's work in assessing the effectiveness of therapy.

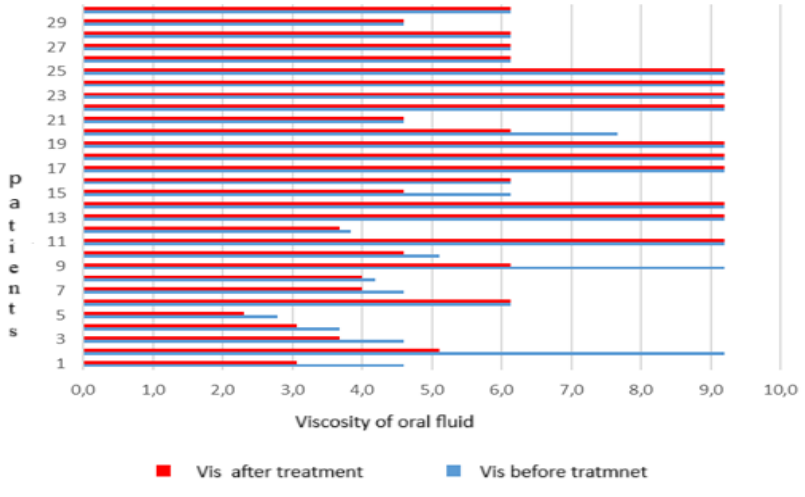


Figure 4. Viscosity of saliva in patients of the comparison group during treatment

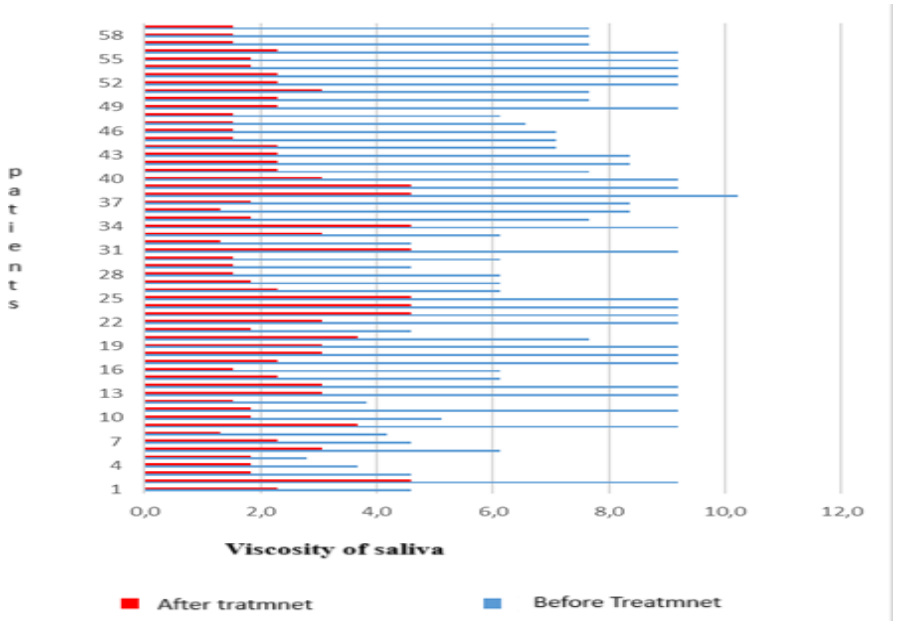


Figure 5. Viscosity of saliva in patients of the main group during treatment

The pH value after treatment shifted to the right, i.e. to an acidic environment in more than half of the comparison group and more than a third of the patients in the main group. The acid-base balance shifted to the left, i.e. to an alkaline reaction in less than a third of the patients in the comparison group and in a fifth of the patients in the main group (Tables 4 and 5).

Table 4. Acid-base balance in patients in the control group

Patients (n=30) of the comparison group			
After treatment		pH range	
Quantity	%		
8	26,67	>7,5	Alkaline
6	20,00	6,8- 7,4	Norm
16	53,33	<6,7	Acidity

Table 5. Acid-base balance in patients of the control group

Patients (n=59) of the **control group**			
After treatment		pH диапазон	
Кол-во	%		
12	20,34	>7,5	Alkaline
27	45,76	6,8- 7,4	Norm
20	33,90	<6,7	Acidity

Thus, the obtained data allow us to conclude that the acid-base balance in patients of the main group in the pH range of "normal" is 45.76%, which is significantly (2.3 times) higher than in the comparison group. Accordingly, in the main group, a decrease in the acidity level of the percentage component among patients is observed 1.6 times less often, in contrast to the comparison group.

Thus, in patients suffering from mucositis against the background of oral cancer, clinically significant changes in salivation parameters are associated with the aggressiveness of chemoradiation therapy. Complex mechanisms of regulation of salivation and its disorders require further research. The features of salivation dysfunction in cancer patients receiving chemoradiation therapy of the oral cavity are a decrease in the volume and rate of salivation, as well as an increase in the viscosity of oral fluid. The use of Laetrile for 10-14 days from the beginning of the course of chemoradiation therapy can significantly prevent a decrease in the volume and an increase in the viscosity of saliva and minimize the associated decrease in quality of life.

To assess the local mechanisms of regulation of immune reactions in patients with mucositis, the content of pro- and anti-inflammatory interleukins was determined in the oral fluid.

The concentration of pro-inflammatory IL-1b in the saliva of patients before the start of cancer treatment - chemoradiation therapy - was 126.14 ± 45.01 pg / ml. The relatively low concentration of anti-inflammatory IL-10 at this time was 94.96 ± 44.20 pg / ml. During this period, dental sanitizing treatment measures were carried out. During the main complex of cancer therapy, mucositis developed, which required local therapy. The concentration of IL-1b at this point in the patients' saliva averaged 303.99±130.18 pg/ml (an increase of almost two times), and IL-10 - 31.68±5.91 pg/ml (a decrease of 50%).

The developed mucositis required local treatment. After the start of local therapy, we took saliva samples at intervals of 1 day.

When using classical therapy, we did not obtain statistically significant differences on days 1 through 7, the fluctuations in values were less than 15–20%, which did not allow us to consider the results significant with such variability.

When using Laetrile to level out inflammation in oral post-radiation mucositis, the concentration of pro- and anti-inflammatory cytokines changes differently. During the first day, the IL-1b concentration decreased, reaching reliable differences by the fifth day. On the 7th day, this indicator began to increase again (Fig. 6, Table 6).

It should be noted that in 4 out of 10 people, the concentration of the proinflammatory cytokine was fundamentally different from the rest of the group. At the same time, when they were excluded from the sample, the trend of changes remained the same. This reflects significant individual characteristics and was the reason for using nonparametric statistical methods.

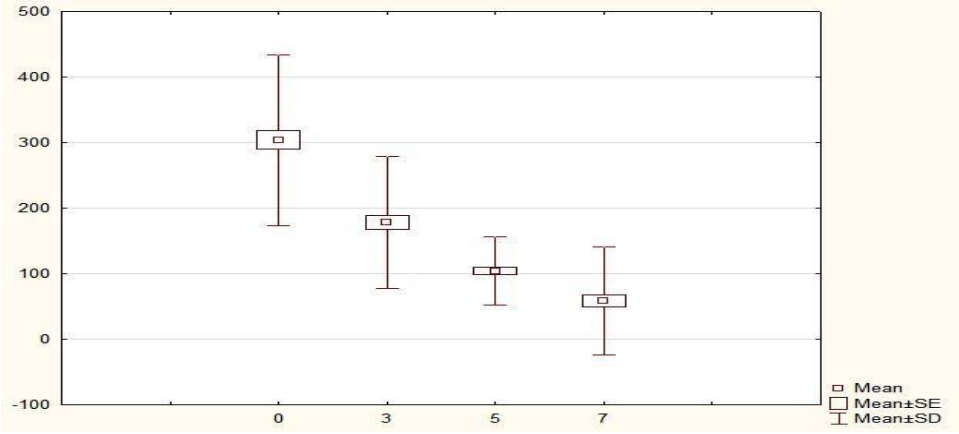


Figure 6. IL-1b concentration (pg/ml) in saliva on days 0, 3, 5, and 7 from the start of Laetrile treatment.

The values on day 5 differ significantly from days 0 and 3 with an error probability of 0.05 according to the Wilcoxon test for dependent samples. The change in the concentration of anti-inflammatory cytokines was generally inverse. Its relatively low concentration before the start of local treatment of oral mucositis was 31.68±5.91 pg/ml, gradually increasing by the 7th day. Three patients also differed significantly in this indicator, in whom IL-10 was significantly lower than in the others (in terms of IL-1b, these patients also had low values, which may be a criterion for exclusion

from the sample). When they were excluded from the sample, the trend did not change and represented a steady increase in the concentration of anti-inflammatory cytokines over time (Fig. 6, Table 6).

Table 6.Mean saliva concentrations in patients treated with Laetrile (M±SD)4.

Marker	Days from the start of treatment			
	0	3	5	7
IL-1b, пг/мл	303,99±130,18	178,57±100,71	104,02±51,86*	58,23±82,72
IL-10, пг/мл	31,68±5,91	111,06±68,95*	215,63±95,82*	311,85±122,31*

From this we can conclude that the existing therapeutic potential of Laetrile has a rapid local effect, reflected in the cytokine profile of saliva, but dynamically and unstable due to the more powerful systemic effect of ionizing radiation and chemotherapy. There is also a significant individual difference in sensitivity to both radiation and local therapy. The clinical and morphological positive data on the effects of Laetrile that we have previously identified make the results convincing in combination and indicate the autonomy of tissue reparative potential and the auxiliary effect of humoral factors in oral fluid.

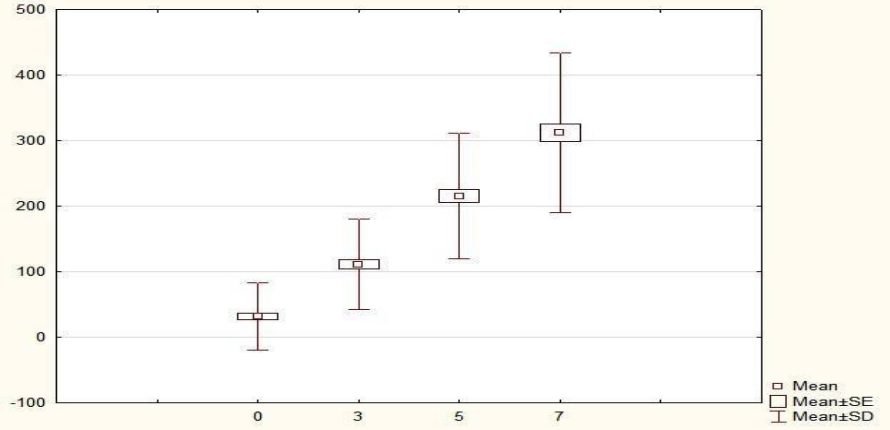


Figure 7.IL-10 concentration (pg/ml) in saliva on days 0, 3, 5, and 7 from the start of Laetrile treatment. The differences between all groups are significant, except for day 5 (no differences from days 3 and 7) with an error probability of 0.05 according to the Wilcoxon test for dependent samples.

The IL-1b/IL-10 ratio before treatment averaged 15-18 units. After treatment, this ratio rapidly declines during the first three days, then the decline slows down, while significantly differing from the control group on days 3, 5, and 7 (Fig. 8).

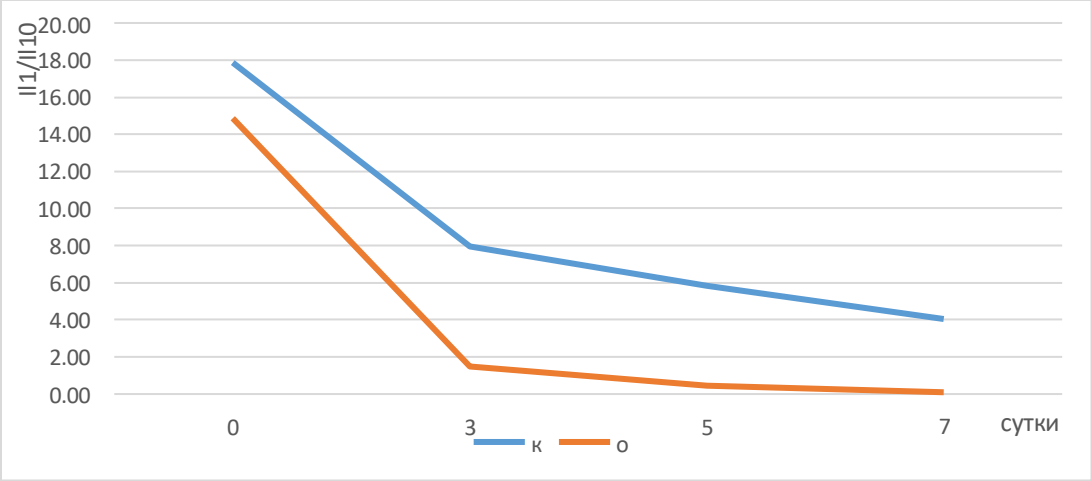


Figure 8. IL-1b/IL-10 M [Q1;Q4] ratio

Thus, Laetrile has a rapid local effect, reflected in the cytokine profile of saliva, which is quite dynamic due to the more powerful systemic effect of ionizing radiation and chemotherapy: the concentration of IL-1b by the 5th day decreases almost threefold, and IL-10 increases almost 7 times. A significant individual difference in the content of these cytokines was revealed, reflecting sensitivity to both radiation and local therapy. From the anamnesis it was revealed that 39 (43.8%) patients suffered from cardiovascular diseases, 32 (35.9%) patients had pathology of the nervous system, 13 (14.6%) had pathology of the thyroid gland, 12 (13.5%) were diagnosed with diabetes mellitus, 4 (4.5%) had pathology of the respiratory system, 3 (3.4%) patients indicated the presence of infectious diseases, two of them had viral hepatitis, and one examined person had tuberculosis and two people indicated occupational hazards.The level of oral hygiene of patients in both groups before and after treatment is presented in Table 7.

Table 7. Level of oral hygiene of patients in both groups

Reference values of the hygiene index	Hygiene Index Assessment	Oral hygiene assessment	Before treatment		Comparisons group		Main group	
					After treatment		After treatment	
			N=89	(%)	N=30	(%)	N=59	(%)
0–0,6	low	good	7	7,87	4	13,33	12	20,34
0,7–1,6	medium	satisfactory	21	23,60	8	26,67	30	50,85
1,7–2,5	high	unsatisfactory	31	34,83	9	30,00	11	18,64
2,6–3,0	very high	bad	30	33,71	9	30,00	6	10,17

Note: N – number of patients

The level of gum inflammation in patients in the comparison group during treatment was different and is presented in Table 7. Thus, the assessment of oral hygiene in patients in the comparison group is significantly worse than in the main group. In particular, the assessment of the hygiene index “very high” in the control group is 30.00%, which is significantly higher than in the main group (10.17%).

Table 8. Level of gum inflammation in patients in the comparison group during treatment

Control group (n 30)								PMA Value %	Degree of damage gingivitis
Before treatment		After treatment							
		Day 3		Day 5		Day 7			
Quantity	%	Quantity	%	Quantity	%	Quantity	%		
3	10	2	6,67	2	6,67	3	10,00	до 25	light
10	33,33	3	10,00	3	10,00	3	10,00	25-50	medium
17	56,67	3	10,00	3	10,00	8	26,67	> 51	heavy

The level of gum inflammation in patients of the main group during the treatment was also different and is presented in Table 9.

Table 9. Level of gum inflammation in the main group during the treatment

Main group (n 59)								PMA Value %	Degree of damage gingivitis
Before treatment		После лечения							
		Day 3		Day 5		Day 7			
Quantity	%	Quantity	%	Quantity	%	Quantity	%		
9	15,25	4	6,78	6	10,17	6	10,17	До 25	light
18	30,51	5	8,47	9	15,25	10	16,95	25-50	medium
32	54,24	4	6,78	8	13,56	7	11,86	более 51	heavy

Thus, severe gingivitis on the 7th day of treatment in the main group is 11.86%, moderate gingivitis was detected in 16.95%. The level of gum inflammation in patients in the main group after treatment is significantly lower than in the control group. This indicates the same tendency to improve the condition of the gums and reduce inflammatory processes in them. The use of Laetrile made it possible to achieve a therapeutic and prophylactic effect in 55 (93.2%) of 89 patients treated with Laetrile while in the comparison group (traditional treatment) in 19 (63.3%) of 30 patients. The effectiveness of the therapy can be conveniently demonstrated figure 9a,b.Patient M., 53 years old, Diagnosis: tongue cancer T3N0M0 Mucositis grade III.



Figure 9. a) – Before treatment **Figure 9. b)** After treatment

Clinical case: Patient I., 58 years old (main group) was admitted to the State Budgetary Healthcare Institution of the Republic of Crimea "V. M. Efetov Crimean Republican Oncology Clinical Dispensary" with a diagnosis of oral mucosa cancer T3N0M0 after the first course of chemotherapy. A few minutes before radiation therapy, a protective device was put on the patient on both parotid areas. After that, distant gamma therapy was performed. SOD 48 -50 Gy. Before irradiation, we used protective mouth guards to prevent the development of radiation sickness of the oral cavity. During treatment (3-4 days), the patient complained of dry mouth, pain when swallowing solid food. Mild mucositis was detected. Tooth mobility of the II degree. Upon examination of the patient, it was revealed that the mucous membrane was slightly hyperemic - edematous. The index assessment of the state of the oral organs is: KPU = 22, GI - 2.5; RMA – 53%. On the 5th day, the patient complained of dry mouth, mild bleeding gums, exposed necks of teeth, pain when swallowing solid food. Regional lymph nodes are normal, oral mucosa is hyperemic, dry, edges of interdental papillae and alveolar processes of the upper and lower jaws are stagnant and edematous, abundant deposits of soft plaque of supra- and subgingival tartar. Periodontal pockets up to 4 mm deep, bad breath. Tooth mobility of the 2nd degree. Single erosive erythematous foci were observed in the oral cavity. Moderate mucositis was detected. On the 7th day, the patient complained of dry mouth, bleeding gums, exposed necks of teeth, pain when swallowing any kind of food. Regional lymph nodes are painful and enlarged, oral mucosa is clearly hyperemic, multiple erosive and ulcerative lesions are observed in the oral cavity, bleeding is noted, the edges of the interdental papillae and alveolar processes of the upper and lower jaws are stagnant and edematous, there are abundant deposits of soft plaque, supra- and subgingival tartar with purulent-hemorrhagic discharge. Periodontal pockets are 6 mm deep, there is an unpleasant odor from the mouth. Tooth mobility of II - III degree. III degree of mucositis was revealed). The status during examination is illustrated in Fig. 10a,b.



Figure 10. Patient I, 58 years old, Diagnosis: oral cavity cancer T3N0M0. a) – Before treatment. b) After treatment.

DISCUSSION

Chemotherapy and radiotherapy are the predominant treatment options for patients with oncological and oncohematological diseases. The use of these two antitumor agents increases the frequency and severity of side effects, which can lead to severe mucositis.

A number of chemotherapeutic preparations and their combinations are used for chemotherapy of malignant neoplasms of the organs and tissues of the mouth. These include bleomycin, cisplatin, carboplatin, fluorouracil, paclitaxel, docetaxel. The most important antitumor drug in the treatment of this localization is cisplatin. Despite the understanding of the causes and mechanisms of oral mucositis, it remains a serious problem for patients receiving treatment for localized head and neck cancer. The severity of mucositis varies greatly and depends on the treatment methods used (total radiation dose, volume and area of irradiation, fractionation regimen, use of chemotherapy) and on the patient-related risk factors (gender, age, lifestyle, presence of carious teeth, concomitant diseases, epigenetic and genetic susceptibility)[34].

Today, several Classification are available to assess the severity of oral mucositis. Staging according to the Radiation Therapy Oncology Group (RTOG) is performed on a scale from I to IV. The first degree includes mucositis that causes mild pain and does not require the use of analgesics[35].

Grade II is typical for the development of focal mucositis or mucositis requiring the use of analgesics.

Grade III mucositis occurs in the form of confluent mucositis or is accompanied by pain syndrome requiring narcotic analgesics.

Grade IV is characterized by the formation of ulcers, areas of necrosis or bleeding.

Oral mucositis associated with treatment in such patients, reduces the quality of life of patients and increases the risk of life-threatening complications To decide on the treatment method for oral mucositis, it is necessary to assess the severity of the disease in a particular patient, which is determined by the methods of prevention and treatment aimed at increasing the patient's tolerance to the treatment and reducing the risk of complications associated with the treatment.

Treatment of oral mucositis is pathogenetic and symptomatic.

There are currently no generally accepted protocols for the prevention and treatment of oral mucositis.

The Mucositis Study Group of the Multinational Association for the Treatment of Cancer (MASCC) and the International Society for the Study of Oral Oncology (ISOO) developed clinical practice guidelines for the treatment of oral mucositis based on a comprehensive systematic review of the literature[36,37] .

From the moment of manifestation of radiation epitheliitis and until the complete subsidence of reactions, the use of wound-healing local agents, irrigation of the oral cavity with an isotonic solution, applications of oils (olive, peach, sea buckthorn or rose hips) to the oral cavity is indicated. In the presence of pain syndrome, local and systemic painkillers are used [38].

Pain relief should be prescribed in accordance with the scale of the World Health Organization. Adequate nutrition and maintenance of water balance are vital. A special role is played by the balance of food intake, which reflects the maintenance of a stable weight. Often, it is necessary to prescribe oral nutritional mixturesAttention is paid to the role of oral hygiene, the use of physical methods (hypothermia, laser therapy), the prevention of infectious complications, and the use of local and general epithelialization-stimulating agents. All of these methods have their positive and negative sides, advantages and disadvantages. Often, the methods are complex, multi-component, require the use of expensive drugs and expensive equipment manufactured abroad.

The goal of treatment of oral mucositis during radiotherapy is to prevent the development of severe oral mucositis and associated secondary infections, as well as to control pain and maintain the ability to eat independently. treatment is carried out with an emphasis on palliative care and symptom control [8]. However, symptomatic therapy does not always achieve adequate pain control, which only emphasizes the importance of developing new treatment strategies[39,40].

Currently, the are considering several groups of means for the prevention of mucositis and treatment of oral mucositis: anti-inflammatory drugs, antimicrobial agents, biological response modifiers, antioxidants, non-pharmacological effects. It is important to note that reducing morbidity may positively impact treatment tolerability, compliance, and quality of life for patients.

Prevention of mucositis mainly comes down to oral hygiene before chemotherapy, visiting the dentist (in the presence of carious teeth and manifestations of periodontitis).

Randomized trials have shown the effectiveness of topical and systemic antimicrobial drugs for the prevention and treatment of oral inflammation[41].

However, antimicrobial drugs also have a number of cytotoxic side effects, which are undesirable in oncological patients who are already receiving high doses of drugs that have an immunosuppressive effect. Therefore, the search for such means and methods that do not have a toxic effect on the oral mucosa and the patient's body as a whole is certainly of scientific interest.

To increase the effectiveness of chemotherapy and chemoradiotherapy for oncological diseases, it is necessary to search for and develop highly effective means for the prevention and/or treatment of various forms of inflammation of the oral mucosa that are affordable, have low toxicity, do not require the use of special equipment, and are suitable for patients both in hospital settings and at home.

The search for methods and means to minimize the impact of an unsanitized oral cavity and effectively prevent mucositis continues to be relevant.

In this work, a herbal preparation was used to increase the effectiveness of methods for preventing and treating inflammation of the oral mucosa in patients. To solve the set tasks, groups of patients receiving radiation or chemoradiation therapy for squamous cell carcinoma of the oropharyngeal region were formed.

A herbal preparation Laetrile was used, the results obtained in the work confirm the effectiveness of the proposed schemes for the prevention and treatment of mucositis in cancer patients against the background of radiation and/or chemotherapy treatment both in clinical (reducing the severity of mucositis and improving the quality of life of patients),

CONCLUSION

The use of Laetrile has reduced the incidence of bleeding gums, erosions and ulcers of the oral mucosa. Its use is recommended for mucositis causing complications chemo- and radiation therapy.

DECLARATIONS

Ethical approval and consent to participate
The work was carried out in compliance with all ethical standards for conducting an experiment and there is a positive decision of the Ethics Committee of the Order of the Red Banner of Labor Medical Institute named after S. I. Georgievsky.

Competing interest

The authors declare that there are no competing interest.

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Authors' contribution

Inessa Romanenko - study design, data collection and analysis, writing the text;

Kristina Arakelyan development of the research methodology and interpretation of the obtained data, preparation of the manuscript

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RESEARCH ARTICLE

PATHOMORPHOLOGICAL CHANGES IN THE MANDIBLE BONE WITH VARIOUS OSTEOREGENERATION METHODS IN THE EXPERIMENT

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ABSTRACT

The article presents a study of pathomorphological changes in the lower jaw bone in rats undergoing osteoplasty of a bone defect in an experiment. The study was conducted on male Wistar rats. Three groups of 20 animals were formed. In the first group, the defect was replaced with bone shavings, in the second - with Kollapan-L material, and in the third - with Kollapan-L with committed autologous mesenchymal stem cells. In the 3rd group of animals, the presence of young connective tissue rich in cells, vascular proliferation and maturation of connective tissue to coarse scar tissue with lymphohistiocytic infiltration were determined, especially at 30-60 days. The Ki67 proliferation index by day 30 of the experiment in the three groups was distributed as follows: 4.93%, 18.2 and 32.8%, indicating a stimulating effect of mesenchymal stem cells. The obtained data indicate acceleration of physiological regeneration under the influence of mesenchymal stem cells committed in the osteogenic direction. The immunomodulatory effect of stem cells allowed to significantly reduce the percentage of purulent infiltrates in the bone defect. Increased expression of proliferation markers Ki67 and angiogenesis markers CD 36 promotes accelerated bone tissue regeneration.

Keywords: bone defect, osteoplasty, artificial hydroxyapatite, biomatrix, mesenchymal stem cells, Ki67, CD38

1. INTRODUCTION

Osteoplasty has been used for many years in orthopedic surgery to restore bone tissue in case of defects or ruptures caused by trauma and tumors, as well as in case of periodontal defects [1-4].

Reconstructive surgery is important to restore the aesthetic and functional role of these complex anatomical areas and can be challenging for both patients and surgeons [5, 6].

Bone regeneration requires that stem cells from both donor and host differentiate into osteoblasts, secrete collagen I and other organic components of the bone matrix, and then mineralize to form fully

functional bone. Previous studies in the literature have used autologous bone grafting, titanium or polyetheretherketone implants, and other modern materials to close bone defects [7].

However, each material has certain limitations, which requires further research to determine the best technique for bone reconstruction in dentistry.

General requirements for an ideal implant include biocompatibility, lack of allergy, radiopacity, cost-effectiveness, ease of use, and sufficient strength [8, 9].

In recent decades, tissue engineering and regenerative medicine have attracted much attention with the expansion of various studies to evaluate biomaterial application strategies for better maxillofacial bone regeneration[10]. Biomaterial scaffolds provide support for cell growth, deliver cellular signals, and have a significant impact on the microenvironment of tissue formation. Various types of biomaterials such as bone grafts, bioceramics, natural and synthetic polymers, and dental derivatives have been clinically applied in the maxillofacial area. However, there are still shortcomings such as incomplete restoration and change in the structure of regenerated bone, complications, or lack of long-term stability [11, 12].

Therefore, it is imperative to clarify the status quo of maxillofacial bone tissue engineering in order to develop more ideal biomaterial scaffolds for maxillofacial bone regeneration that will completely restore the normal physiological functions of the defect areas. Over the past threedecades, the use of stem cells delivered with various scaffolds or growth factors for bone tissue engineering has made enormous progress[13].

Many types of stem cells have been isolated from various tissues for use in bone tissue engineering [14, 15]. Currently, the use of various postnatal stem cells, including bone marrow mesenchymal stem cells 16, muscle stem cells, adipose stem cells [18], dental pulp/periodontal ligament stem cells [19, 20], periosteum stem cells [21], umbilical cord stem cells [22], peripheral blood stem cells [23], urinary stem cells [24], apical papilla stem cells, and induced pluripotent stem cells, for bone tissue engineering and repair has been widely applied in clinical practice [25]. Despite extensive preclinical studies using many different stem cells from diverse tissue resources, the clinical application of stem cells is still limited due to the fact that most of them require culture and expansion. Ideally, stem cell-based strategies in bone tissue engineering require stem cells from both donor and host to differentiate into osteoblasts, secrete type I collagen and other organic components of the bone matrix, and then mineralize to form fully functional bone.

Therefore, the development of new methods or devices that allow the isolation of stem cells at the point of care for the repair of bone defects or the repair of non-union fractures is of critical importance.

The aim of the study was to study the pathomorphological changes in the mandibular bone of rats during osteoplasty of a bone defect in an experiment.

2. MATERIAL AND METHODS

The study was conducted on 60 male Wistar rats weighing 300±30 g. Osteodamage in animals was induced by drilling a defect with a dental bur in the area of attachment of the masticatory muscles in the angle of the lower jaw (2 mm in diameter). Then, for group 3, Kollapan-L granules were immersed in a MSC culture for 12 hours and the bone defect was filled.

We formed 3 groups of 20 animals each:

- group 1(control) - bone sawdust obtained during the application of the defect was placed into the bone defect;
- group 2 - the defect was filled only with Kollapan-L No. 1 mechanically crushed five times, thus obtaining fragments of 0.2 cm;
- group 3 - the bone defect was filled with Kollapan-L No. 1 with committed autologous mesenchymal stem cells.

In all groups,the mucosal defect was sutured with 4/0 Vicryl thread over the defect with plastic material. Bone marrow (BM) explantation was performed under aseptic conditions from the sternum or iliac crest (crista iliaca) in an amount of 20-40 ml with the addition of 625 units/ml heparin (Darnitsa, Ukraine). BM aspirate was layered on a Histopaque-1077 gradient, density 1.077 g/ml, (Sigma, USA) and centrifuged for 30 min. at 1500 rpm. The resulting mononuclear cells were collected with a pipette and successively washed 3-4 times in Hanks' solution (Biolot, Russia) by centrifugation at 1000 rpm for 14 min. The resulting mononuclear bone marrow cell suspension was seeded onto collagen-coated 75 cm2 culture flasks (Corning-Costar, USA) at a concentration of 2–5×106 cells per flask. MSCs were cultured in a mixture of DMEM/F12 nutrient medium,

1:1, (Sigma, USA) with the addition of 10% fetal calf serum (Biolot, Russia), 0.75 mg/ml glutamine (Institute of Poliomyelitis and Viral Encephalitis, Russia), 2 ng/ml basic fibroblast growth factor (Sigma, USA) and 100 U/ml penicillin and streptomycin (Darnitsa, Ukraine) in a CO2 incubator (Jouan, France) at 37ºC and 5% CO2 atmosphere. The medium was changed every 3-4 days of cultivation. The cultures reached the primary monolayer on the 8th-11th day of cultivation, depending on the seeding density of the primary isolated cell suspension, individual characteristics of the donors and the level of proliferative activity of the cells. Passaging, or subculturing, was carried out using a mixture of trypsin/EDTA solutions (Biolot, Russia) in a ratio of 0.05%:0.02% in PBS, pH 7.4 (Sigma, USA).

The passaging ratio was 1:2 or 1:3. After that, the cells were cultured in a CO2 incubator under the same conditions. As a result of these manipulations, an uncommitted cell culture of MSCs was obtained. After the uncommitted MSC cultures reached a confluent monolayer, they were incubated in an osteogenic growth medium containing DMEM/F12, 1:1, (Sigma, USA) supplemented with 10% fetal bovine serum (Biolot, Russia), 0.75 mg/ml glutamine (Institute of Poliomyelitis and Viral Encephalitis, Russia), and an osteogenic supplement - 50 μg/ml L-ascorbic acid (Sigma, USA), 10 mM β-glycerophosphate (Sigma, USA), 0.1 μM dexamethasone and 100 units/ml penicillin and streptomycin (Darnitsa, Ukraine) in a CO2 incubator (Jouan, France) at 37ºC and 5% CO2 atmosphere. The growth medium was changed every 3-4 days of cultivation (Fig. 1).

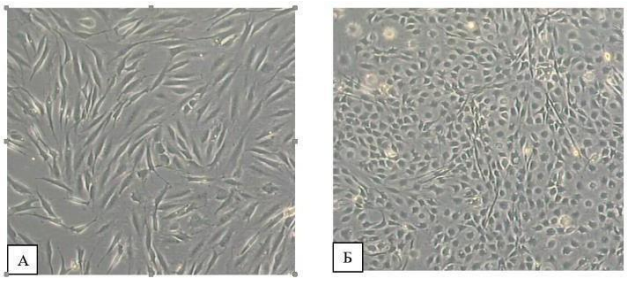


Figure 1. MSC culture (A) and after scaling (B) (FKM X200).

After 30 days, a standard histological examination of the tissues of the defect site was performed. At the 10th, 30th and 60th days, the proliferation marker KI-67 and the angiogenesis marker CD 34 were determined using immunohistochemical methods. Mouse antibodies from Cellmarque, USA, were used to study CD 34, and rabbit antibodies (BioVitrum, Russian Federation) were used for Ki-67.

Statistical methods. The Shapiro-Wilk test (W) was used to check the data distribution for normality, which made it possible to use it even with a small sample (n<30). To identify significant differentiations between the average values of different populations of comparable groups, variation statistics methods were using Student's t-test with Bonferroni correction for multiple comparisons with a type I error probability of p=0.05. The data were considered reliable at p<0.05. Results. The effect of osteoplastic materials modified with culture supernatant on the experimental bone wound was carried out by comparing the regeneration indices in relation to groups 1 and 2. Microscopy of histological preparations of animals on days 7-10, where traditional bone sawdust (group 1) and Kollapan-L (group 2) were used, the following patterns were revealed. In sections, in 35.6% of cases, in group 2, mild purulent inflammation was noted (Fig. 2).

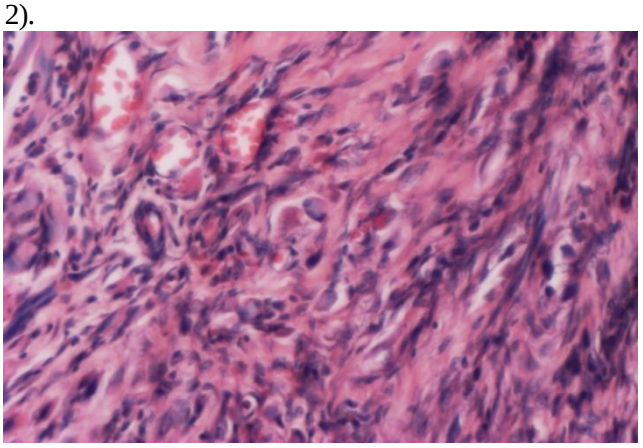


Figure 2. Osteoblastic granulation tissue with infiltration of lymphocytes, macrophages and neutrophils in group 1 on day 30 of the experiment

In comparison with the control group, where severe purulent exudate was detected in 52% of cases. In 78% of cases, osteotropic material was observed in the bone defect in group 3. In the area of the bone defect, the presence of both young connective and scar tissue was revealed, which indicated the onset of bone tissue regeneration (Fig. 3).

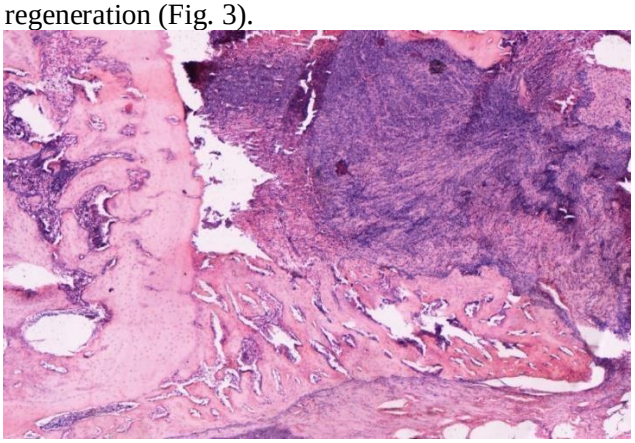


Figure 3. Active maturation of osteoblastic granulation tissue with the formation of spongy bone in group 2 on day 30 of the experiment.

Signs of bone regeneration were more pronounced in groups 2 and 3, compared to the control. Regeneration was manifested in the proliferation of numerous cells with round, oval nuclei, dispersed chromatin, most likely proliferating osteoblasts, pronounced proliferation of osteoblasts and the formation of small bone beams in 34.8% of cases (Fig. 4).

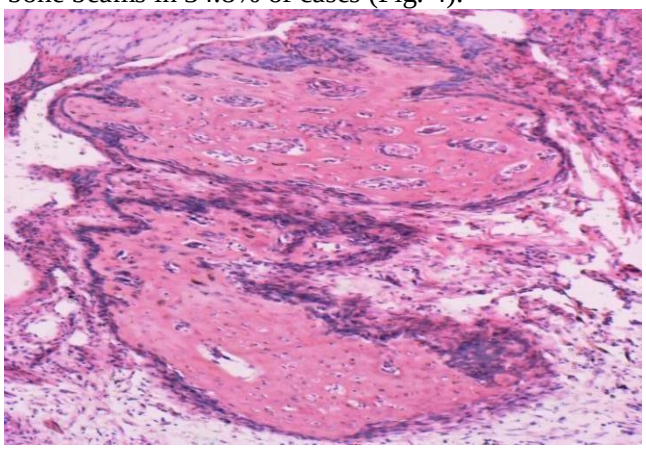


Figure 4. Active formation of bone trabeculae against the background of mild inflammatory infiltration on the 30th day after using Kollapan-L with committed mesenchymal stem cells. Hematoxylin-eosin, magnification 15. In the 1st group, insignificant proliferation of osteoblasts was noted, with the formation of osteoid in 18.2% of cases. Thus, when using Kollapan-L under experimental conditions, an osteoconductive effect was noted in the area of the bone wound and an osteostimulating effect manifested in the form of pronounced proliferation of mature forms of osteoblasts and the formation of small bone beams in 45% of cases.

In the 3rd group of animals, where Kollapan-L with committed stem cells was implanted, the wound showed the presence of young connective tissue rich in cells, proliferation of vessels and maturation of connective tissue to coarse scar tissue with lymphohistiocytic infiltration, especially at 30-60 days (Fig. 3).

Bone regeneration was expressed in the presence of active (proliferating) bone marrow in the bone marrow empty spaces of the pre-existing bone and the proliferation of osteoblasts. In the 1st group, regenerative changes were noted, expressed in the formation of bone marrow cavities with the presence of young connective or osteogenic tissue.

The Ki67 proliferation index by the 30th day of the experiment in three groups was distributed as follows: 4.93%, 18.2 and 32.8%, respectively, which indicates the stimulating effect of mesenchymal stem cells in osteoplastic operations (Tables 1, 2). The reduction of the purulent process confirms the immunomodulatory effect of stem cells. The increase in CD34 content in the three groups was on day 30 in group 1 from 10.56±1.8 to 13.48±21, on day 60 to 14.22±12, in group 2 from 11.23±2.2 to 14.33±1.7 and 15.8±1.4, in group 3 from 12.23±1.3 to 15.1±2.2 and 16.4±1.1, respectively, which indicates the participation of angiogenic factors in the physiological bone regeneration of the bone defect of the lower jaw.

Table 1 Dynamics of Ki67 in groups at different times

Groups	10-day	30-day	60-day
1	7,96±1,89	9,11±2,18	10,11±2,41
2	9,26±3,47*	12,58±2,21*	14,22±1,37*
3	11,12±2,25#,@	18,46±2,11#,@	24,21±1,98#,@

Note: * - the difference between the indicators of groups 1 and 2 is reliable ($p<0.05$); # - the difference between the indicators of groups 2 and 3 is reliable ($p<0.05$), @ - the difference between the indicators of groups 1 and 3 is reliable ($p<0.05$).

Table 2 Dynamics of CD34 in groups at different times

Groups	10-day	30-day	60-day
1	10,56±1.8	13,48±21	14,22±12
2	11,23±2.2*	14,33±1,7*	15,8±1,4*
3	12,23±1,3#,@	15,1±2,2#,@	16,4±1,1#,@

Note: * - the difference between the indicators of groups 1 and 2 is reliable ($p<0.05$); # - the difference between the indicators of groups 2 and 3 is reliable ($p<0.05$), @ - the difference between the indicators of groups 1 and 3 is reliable ($p<0.05$).

4. DISCUSSION

The problem of stimulating physiological tissue regeneration raises a number of controversial aspects that require study. To eliminate these issues, bone tissue engineering is widely studied with the combination of three main components: osteogenic cell resources, biomaterials and signaling molecules [26]. The authors analyze osteogenic tissue reparation using several types of plastic material, firstly, these are the patient's own bone chips, secondly, this is Collapan L and thirdly, Collapan L with its own autologous mesenchymal stem cells. The stem cells used in the experiment were committed, i.e. targeted to differentiate into bone tissue, this was done in order to accelerate the process of bone tissue regeneration and mesenchymal stem cells did not take the shortest path of differentiation into connective tissue. When studying the physiological regeneration characteristics of the lower jaw bone tissue in rats under the influence of mesenchymal stem cells on the jaw bone injury model, it was demonstrated that osteogenic differentiation of connective tissue cells and bone formation in Group 3 were more vigorous compared to other groups. This was confirmed by the concentration of Ki67 and CD 34, which demonstrated acceleration of angiogenesis and tissue proliferation. To study the question whether bone restoration stimulated by co-implantation of Kollapan-L with mesenchymal stem cells is mediated by increased proliferation in defective bone tissues, we determined the number of cells expressing Ki67, a proliferation marker, using immunostaining. In previously published works devoted to bone tissue regeneration, this marker was characterized as an objective criterion [27].

The number of Ki67-positive cells showed a slight increase after implantation in groups 1 and 2 compared to group 3. It turned out that combined implantation of Kollapan-L with mesenchymal stem cells increased the expression of Ki67-positive cells severalfold.

The integrity of bone, as a highly vascularized tissue, depends on angiogenesis and close communication of bone cells in time and space. Therefore, sufficient angiogenesis plays a key role in the development and restoration of bone. Local vascularization in the early stages after transplantation provides various necessary nutrients for the activity of osteogenesis, but also plays an irreplaceable role between the bone and adjacent tissues and organs [28].

An increase in CD34 expression in group 3 patients indicated the activation of angiogenic factors of bone tissue regeneration. In our previous studies, we confirmed that committed MSCs actively produce cytokines – IL-1β, IL-2, IL-6, IL-8, FNP-α and IL-4[29]. During osteogenic differentiation, the production of IL-2, IL-6 and IL-8 significantly increases. Thus, it was proven that mesenchymal stem cells, being their own building material, stimulate regeneration, angiogenesis and enhance the synthesis of sulfated multipolysaccharides, which together lead to activation of bone tissue regeneration. In addition, arachidonic acid derivatives secreted by mesenchymal stem cells lead to both local and systemic immunomodulation, resulting in a decrease in the number of purulent complications and inflammation. The advent of bone tissue engineering technology has stimulated significant progress in the study of new materials and has significantly contributed to increasing the effectiveness of bone defect treatment. However, low-bionic materials, poor biocompatibility, cell migration, adhesion and proliferation still remain problems. Precise control of cell differentiation, gene and growth factor expression, and safety of clinical application need further study [30]. Through cross-integration and development of interdisciplinary concepts and technologies in the field of bone repair, such as cell biology, immunology, material science and manufacturing, bioactive bone-like tissues can be created by searching for and developing new substitutes for bone repair or directly in vitro. It is expected that regenerative restoration of bone defects will be widely used in clinical practice in the near future.

CONCLUSION

Thus, our data indicate the acceleration of physiological regeneration under the influence of mesenchymal stem cells committed in the osteogenic direction. The immunomodulatory effect of stem cells made it possible to significantly reduce the percentage of purulent infiltrates in the bone defect. An increase in the proliferation markers Ki67 and angiogenesis marker CD 36 leads to a favorable acceleration of bone tissue regeneration.

DECLARATIONS

Ethical approval and consent to participate
The work was carried out in compliance with all ethical standards for conducting an experiment and there is a positive decision of the Ethics Committee of the Order of the Red Banner of Labor Medical Institute named after S.I. Georgievsky (protocol No. 5 dated 05/18/2024).

Competing interest

The authors declare that there are no competing interest.

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Authors' contribution

I. G. Romanenko - study design, data collection and analysis, writing the text; V. Yu. Mikhailichenko - development of the research methodology and interpretation of the obtained data, preparation of the manuscript; N. L. Kulikova - protocol development, study implementation, analysis of the obtained data;
D. S. Parshin - writing the text, scientific editing of the manuscript

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RESEARCH ARTICLE

MEDICAL AND SOCIAL ASPECTS OF HEMOCONTACT INFECTIONS IN DENTAL INSTITUTIONS OF OSH REGION, KYRGYSTAN

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ABSTRACT

Background: Public health is at risk by the spread of hemocontact diseases such HIV, viral hepatitis B and C, especially in medical and dental facilities where rigorous infection control procedures are very essential. Transmitted via blood contact, these infections pose an extreme risk for patients undergoing dental treatment as well as for healthcare professionals as contact with blood and other fluids is frequent.

Objective: This research sought to assess the risk of infection among medical staff and patients at dental facilities in the Osh area of Kyrgyzstan as well as the frequency of infections passed via a hemocontact mechanism. To guarantee strong and multifaceted findings, a complete strategy combining epidemiological, analytical, sociological, laboratory, and statistical techniques was used.

Method: The study consisted of statistical, laboratory, social, and epidemiological techniques. Along with lab test findings, data sources included information on the incidence of HIV, HBV, and HCV (2020–2024) from the Osh Center for Disease Prevention. Developed to evaluate hygienic conditions, risk factors, and health awareness among 256 dentists, residents, and 220 dental students was a specifically customized questionnaire. Using MS Excel and Epi-Info tools, data were examined; the Student's t-test followed to determine statistical significance.

Result: The results showed that while viral hepatitis B, hepatitis C, and HIV are a significant public health problem, the epidemiological condition of hemocontact infections in the Osh region is still severe. Many times, dental offices overlook hygienic and epidemiological recommendations; disparities in infection control practices during patient treatment clearly increase the danger of viral spread. This risk is significantly increased by factors like poor sterilizing of tools, low compliance with infection safety guidelines, and holes in preventive precautions.

Conclusion: Strict use of a set of policies is very necessary to reduce the spread of hemocontact infections in dental treatment environments. This includes enforcing adherence to infection prevention and control criteria, enhancing monitoring systems, and guaranteeing thorough instruction for dental staff on infection safety methods. By closing these gaps, one may improve the safety of dental settings, therefore shielding patients from hemocontact infections as well as healthcare personnel.

Keywords*:*Hemocontact infection, dental infection prevention, bloodborne disease epidemiology, healthcare-associated infections, sterilization and disinfection.

1.INTRODUCTION

Hemocontact infections (HCI), including viral hepatitis B (HBV), hepatitis C (HCV), and human immunodeficiency virus (HIV) are social and great economic interest implications because these diseases are 10 of the main causes of death from infectious diseases. The term blood ‘hemocontact containing

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infections' fluids, refers either to direct or indirect with blood or other bodily fluids. The incidence of HCI has grown over the past year in dentist clinics among other healthcare workplaces. Strict infection control policies and preventative techniques are desperately needed in healthcare contexts because patients, medical personnel, and medical students engaged in clinical practice run more risk of exposure [1].

One of the most often used healthcare treatments continues to be dental treatment. But the rising prevalence of blood-borne diseases such HIV, HBV, and HCV puts patients and dental professionals somewhat under concern. The potential spread of these diseases is facilitated by insufficient adherence to infection control policies in dentistry clinics.

The increased HIV pandemic, especially among high-risk populations like injecting drug users, has resulted in its spread in hospital environments including a confirmed outbreak among the juvenile population in Southern Kyrgyzstan. This scenario emphasizes the ongoing risk of hemocontact virus transmission in medical settings and calls for quick action to improve infection control policies in dentistry clinics [2].

Using the Osh area of the Kyrgyz Republic as a case study, this research intends to examine the frequency of hemocontact infections and evaluate the related infection risks among patients at dental facilities and healthcare staff.

The research aims to provide evidence-based suggestions for improving infection prevention strategies and preserving public health inside oral healthcare institutions by identifying areas of weakness in infection control procedures.

2.1 Study Design

The study aimed to identify the risk factors for hemocontact infections (HCI) at dental institutions, assess the awareness of infectious safety among dental professionals and students, and investigate the frequency of HCI among the Osh area of the Kyrgyz Republic population. Data were gathered and examined using a mix of social, epidemiological, laboratory, and statistical approaches.

2.2 Study Objects and Subjects

The research focused on dental groups comprising Osh State University academics majoring in dentistry, residents, practicing dentists, and students. The research included the degree of awareness on infectious safety, the results of a sociological survey on the risk factors for HCI infections at dental facilities, and the general frequency of HCI among the population.

2.3 MATERIAL AND METHODS

The study utilized several data sources to assess the incidence and prevalence of HCI in the Osh region:2.3.1 Add population Hepatitis B (HBV) and Hepatitis C (HCV) incidence statistics from the Osh Center for Disease Prevention and State Sanitary and Epidemiological Department for the years 2020–2024. 2.3.2 Results of laboratory tests for HBV and HCV carriers from Report Form No. 4-1, thereby illuminating the state of population infection status.2.3.3 Information on the status of HIV infection registration for the same time (2020–2024) gathered from the Osh Regional Center for the Prevention of Hemocontact Infections and HIV.**2.4 Survey Development**

A customized survey was designed to evaluate the knowledge level of dental professionals and students as well as to identify the risk factors of HCI infection in dental clinics. The survey consisted on three main sections: 2.4.1 Sanitary and Hygienic Conditions in Dental Clinics: Questions centered on the current safety guidelines, infection control policies, and hygienic practices used in dental establishments.

2.4.2 Risk Factors for HCI Infections: This part sought to find the general knowledge of dental staff on routes of transmission, preventative actions, and general awareness of the risk factors for infection with HCI viruses, including HIV, HBV, and HCV.

2.4.3 Self-Assessment of Health: The last half consisted of questions about personal health condition and participants' evaluation of their own risk of acquiring HCI viruses.

2.5 Study Population

The sample size consisted of 256 dentists and residents from dental clinics in the Osh area, as well as 220 students from Osh State University's "Dentistry" department in their fourth and fifth years. All participants were chosen based on their experience providing direct patient care in dental settings or participating in dental education.

2.6 Data Collection

At Osh State University's Department of "Public Health" and "Therapeutic Dentistry," the questionnaires were distributed to students during their free time outside of the usual course of instruction. Every participant signed a consent form, and the survey was carried out in line with ethical protocols.

2.7 Epidemiological Data

The infection burden in the region was assessed using epidemiological indicators, including the incidence of HBV, HCV, and HIV infections per 100,000 individuals and the prevalence of viral hepatitis markers (HBV and HCV) per 1,000 individuals examined.

2.8 Statistical Analysis

The Epi-Info software program and MS Excel were employed to process the sociological survey results, as well as the collected data on morbidity, HBV, and HCV carriers. The data was summarized using descriptive statistics, which included mean values (M±m). The validity and accuracy of the findings were guaranteed by evaluating the reliability of the results using the Student's t-test (t) and the error-free forecast criterion (P).

2.9 Ethical Statement

The principles of the Declaration of Helsinki were adhered to in the conduct of this investigation [3]. The IRB waived the requirement for ethical approval for the study, as all data were anonymized and collected in accordance with the ethical standards of observational research, owing to its retrospective nature. The study was conducted with the utmost confidentiality for all participants.

3.RESULTS AND DISCUSSION

Regarding hemocontact infections—more particularly viral hepatitis B and C-as well as HIV, the epidemiological condition in the Osh area of Kyrgyzstan still causes considerable concern. Transmitted via blood or blood-containing secretions, these diseases remain an immediate risk to the general community as well as to healthcare professionals [4]. The ongoing frequency of these diseases in the society, especially in dental environments, requires prompt attention to infection control strategies and preventative actions [5]. The Osh Center for Disease Prevention and State Sanitary and Epidemiological Supervision reports that HIV and viral hepatitis B and C are common in the region. These diseases are common in the general population and affect medical professionals, including dentists, who are at risk from blood exposure during operations. Table 1 shows the average yearly occurrence of various illnesses, underscoring the need for improved healthcare and community prevention. The annually registration of viral hepatitis B and C cases stresses the need to investigate epidemiological trends and risk factors. The research shows that the prevalence remains high across demographic categories, with medical staff, notably dentists, who typically operate in bloody conditions, being particularly vulnerable. Thus, strong infection control methods in dentistry clinics and other healthcare institutions are needed to reduce HCI spread.

Table 1. Dynamics of viral hepatitis incidence in Osh region (per 100 thousand population)

Year	Acute hepatitisT				Chronic hepatitisT			
	B		**C**		**B**		**C**	
	Indicator ±m Indicator	±m	Indicator ±m Indicator	±m	Indicator ± m Indicator	±m	Indicator ± m Indicator	±m
2020	1,7	±0,4	0,9	±0,22	34,2	±1,7	11,0	±09
2021	2,4	±0,4	0,4	±0,2	30,2	±1,6	10,8	±09
2022	2,7	±05	1,2	±0,3	33,9	±1,7	11,1	±09
2023	2,4	±0,4	1,9	±0,4	36,1	±1,6	12,6	±1,0
2024	1,7	±0,4	1,2	±0,4	39,4	±1,8	14,1	±1,1

Significant trends in the incidence of acute and chronic viral hepatitis B and C in the Osh area across the study's period are presented by the statistics shown in Table 1. Particularly, with an average of 1.7 ± 0.4 per 100,000 persons, the incidence of acute viral hepatitis B was very constant. From 0.9 ± 0.22 per 100,000 in 2020 to 1.2 ± 0.3 per 100,000 in 2024 ($p > 0.005$), the incidence of hepatitis C did, however, exhibit a clear rising trend. This spike in hepatitis C infections points to a worrying general load of this virus in the area. The chronic types of hepatitis also show this kind of tendency. Suggesting a larger long-term prevalence of chronic infection, the intense incidence rate of chronic hepatitis B considerably increased from 34.2 ± 1.7 per 100,000 in 2020 to 39.4 ± 1.8 per 100,000 in 2024 ($p < 0.005$). In same vein, the prevalence of chronic hepatitis C rose from 11.0 ± 0.9 in 2020 to 14.1 ± 1.1 per 100,000 in 2024. During the research period, the ratio between acute and chronic types of hepatitis shows significant new perspectives on the course and identification of these illnesses. Hepatitis B had a 1:12 acute to chronic case ratio; Hepatitis C had

a 1:9. The asymptomatic nature of acute hepatitis, often leading to delayed diagnosis and a higher incidence in the chronic phase, likely accounts for this discrepancy. Delayed awareness of acute hepatitis adds to the continuous spread of the illness as people stay ignorant of their infection and keep acting in ways that help the disease to spread. Apart from the general population trends, laboratory investigations revealed the pervasive existence of viral hepatitis markers among the general population as well as among medical professionals [6].

Table 2 shows that from 2020 and 2024, the general population's 1.5 times the incidence of hepatitis B shows increases. From 29.3 ± 0.8 in the year 2020 to 45.3 ± 1.1 in the year 2024, the frequency of chronic hepatitis B carriers per 1,000 persons climbed—a concerning rise ($p < 0.005$). This implies that, in spite of current public health campaigns meant to stop the hepatitis B virus, it is still transmitted constantly [7]. The incidence of hepatitis B and C symptoms did not vary substantially during the research period among healthcare staff members with a high occupational risk for hemocontact infections. The indications for Hepatitis B were about 19.7 ± 1.4 per 1,000 individuals in 2020, thereafter decreasing to 20.1 ± 1.4 in 2024. The prevalence of hepatitis C indicators remained stable, with a little increase from 10.7 ± 1.4 per 1,000 in 2020 to 11.2 ± 1.0 per 1,000 in 2024. The findings indicate that, despite the execution of infection control techniques like vaccination, the incidence of hepatitis B and C infections among healthcare personnel persists at a notably elevated level.

Moreover, among medical professionals, the incidence of hepatitis B and C is around 1.5 to 2 times less than in the general population ($p < 0.005$). This variation might be ascribed to the consistent use of immunization campaigns aimed at high-risk healthcare professionals and infection control measures [8 9].

Notwithstanding this lower frequency, the persistent presence of hepatitis markers among healthcare professionals emphasizes the need of continuous infection control strategies, including frequent screening, vaccination, and strict adherence to hygienic practices, to further lower the risk of transmission in healthcare environments [10].

Table 2. There areельonly a few markers of viral hepatitis B and C among the tested population, including medical personnel with a high occupational risk of infection in the Osh region (per 1000 examined people)

Years	InGY				VGC			
	Total population		Health workers		Total population		Health workers	
	Ad impressions Indicator	±m		±m	Ad impressions Indicator	±m		±m
2020	29,2	±0,8	19,7	±1,4	16,5	±06	10,7	±1,1
2021	34,9	±0,9	17,5	±1,3	20,1	±0,7	11,1	±1,0
2022	38,1	±1,0	15,9	±1,3	15,7	±0,6	10,0	±1,0
2023	43,3	±06	18,1	±1,2	14,1	±06	8,5	±09
2024	45,3	±1,1	20,1	±1,4	16,7	±0,7	11,2	±1,0

3.1 Epidemiological Situation of Hemocontact Infections in the Osh Region

The Osh region's epidemiological condition on hemocontact infections especially HIV remains alarming. Official statistics showing the overall number of HIV-infected people in the area as of January 1, 2024 show 1,006 (30.9%) cases linked to injectable drug users and 386 (11.9%) cases among children under 14 years of age. Two main periods—2002–2017, when injecting drug users were disproportionately affected due to the lack of adherence to precautionary measures during drug use; and 2007–2009, when a hospital outbreak resulted in HIV transmission among children in the healthcare facilities of the region—significantly contribute to the spread of HIV via the hemocontact route in the region. This continuous HIV pandemic draws attention to the stressful epidemiological setting for infectious illnesses spread in the Osh area via hemocontact pathways. Such illnesses are very common among the general population, which increases the danger of cross-contamination especially in medical facilities [11].

Given the nature of the operations involved, these establishments including dentistry offices have more chance of spreading among patients and staff. Particularly dental offices, the healthcare environment is classified as a high-risk place for the dissemination of hemocontact diseases like HIV, hepatitis, and other bloodborne viruses [12].

3.2 Growing Dental Care Infrastructure and Its Challenges

Kyrgyzstan's healthcare system, especially the dentistry field, has seen major changes over the last three decades that have resulted in a fast rise in the count of dental establishments. With a staffing ratio of 1.9 physicians and 3.3 secondary medical workers per 10,000 inhabitants, there are now nine state-run dental clinics in the Osh area and city manned by 310 doctors and over 540 medical professionals. The area also includes over 250 private dentistry offices, centers, and clinics. Although this growth has enhanced dental care availability, it has also presented certain difficulties for infection management. The number of dental offices has grown in line with changes in elements that help nosocomial infections—especially those spread by hemocontact—to proliferate. Dental offices naturally include treatments that expose one to bloodborne diseases, hence the lack of rigorous infection control policies increases the danger of transmission. Given the state of the region's healthcare system, which suffers notable flaws in infection control, this is especially alarming [13].

3.3 Infection Control Challenges in Dental Clinics

Examining infection control policies holistically throughout Osh region's dentistry offices finds various serious flaws and noncompliance. Six (66.6%) of the nine state dental offices in the area still occupy modified or converted buildings without centralized hot water supply systems, out of which The current water heaters are not adequate for the demand; many clinics lack centralized sanitation; only the dental clinics in Osh and Karasu districts have working centralized sewage systems. In some clinics, the water supply does not reach every part of the clinic and the sewage systems are sometimes local and inadequate [14]. Furthermore adding to a dangerous and hazardous working environment are numerous clinics without appropriate ventilation or inadequate maintenance of current systems. Furthermore, the actual locations of the dentist offices fall short of the necessary hygienic guidelines. Whether surgical, orthodontic, or general, the working space in dental offices is often insufficient. Many clinics provide just 9-10 square meters instead of the advised 14 square meters per workstation, therefore increasing the risk of cross-contamination and so compromising the general safety of patients and healthcare personnel. In dental environments, these architectural flaws and deviations from infection control procedures provide a substantial risk for the spread of infectious illnesses. This emphasizes how urgently better rules, control, and infrastructure changes are needed to stop hemocontact diseases from spreading among the dental clinics in the area [15].

3.4 Infection Control Deficiencies in Dental Clinics of Osh Region

A comprehensive assessment of Osh area dental clinic infection control policies has shown notable shortcomings in sterilizing procedures and hygienic standards compliance. Out of all the current dental offices, only three have centralized sterilizing departments completely compliant with legal requirements. On the other hand, most clinics (58.9%) run sterilizing facilities in specially designed rooms, where the current sterilizing tools are antiquated and frequently degraded due to wear and tear impacting around 35–40% of the equipment. Under 26.5% of cases, sterilization operations are contracted out to family medical centers and territorial hospital Central Sterilization Offices (CSOs). Nonetheless, an evaluation of these CSOs reveals that more than 52.2% of them fall short of the set Infection Control Regulations (ICR) regarding space requirements, industrial premises, and availability of current sterilizing equipment [16] .

The execution of strict anti-epidemic measures within working spaces determines much of the prevention of healthcare-associated infections (HAIs) in dentistry clinics. Still, following basic infection control guidelines is not perfect. Only 54.5% of instances saw pre-sterilization cleaning of medical devices and the use of disinfection and cleaning policies based on zoning concepts that is, separation of "clean" and "dirty" regions. With infractions recorded in up to 63.8% of the assessed institutions, the general non-compliance with hand hygiene standards among medical staff raises very alarming results. Many medical professionals showed poor understanding of correct hand washing methods and the appropriate antiseptic usage. Furthermore aggravating the danger of cross-contamination are the lack of basic items as disposable paper towels, cloth napkins, and antiseptic dispensers. The inadequate supply of personal protection equipment (PPE) is another important flaw noted in dentistry offices [17]. On a per-patient basis, medical staff members often lack necessary protective gear like disposable gloves, safety goggles, and waterproof aprons. This often leads to PPE reuse, which greatly raises disease transmission risk. Moreover, the absence of automated washing and disinfection devices in dental offices results in ongoing dependence on hand cleaning of used dental tools. Healthcare professionals who manually handle instruments run an occupational risk as pre-sterilization processes increase their contact to infectious pathogens [18].

Furthermore compromising the efficacy of infection control strategies in these facilities is the lack of current sterilizing technology like steam and low-temperature sterilization techniques. The results highlight how urgently sterilizing infrastructure has to be modernized and how closely infection control policies should be followed to guarantee patient and healthcare worker safety in dental clinics all over the Osh area. Examining dental clinics in the Osh area and their Dental Sterilization Services (DSS) has shown notable non-compliance with legal requirements. Deficiencies in terms of space and infrastructure, breaches of basic anti-epidemic criteria, inadequate artificial ventilation, and the lack of centralized hot water supply define the shortcomings. These elements undermine patient comfort and safety as well as medical staff working conditions. These flaws therefore provide an environment fit for the development of nosocomial infections (healthcare-associated infections, HAIs), including hemocontact diseases like viral hepatitis and HIV [19].

Of the dentists who responded, 56.4±3.1% said their health was "good," 21.6±2.5% said it was "satisfactory," and 12.0±2.0% said it was "poor." In addition, 75.2±2.6% of respondents said they were tired at the conclusion of their work shifts; 36.5±3.2% mentioned work overload; and 29.0±2.8% said rest intervals between patient visits were absent. The poll also showed that polyclinics lacked basic conveniences such rest spaces and facilities for physical activity. Strict attention to preventative actions is very essential considering the higher risk of HAIs in dental environments. Ensuring compliance with infection control policies depends critically on the awareness and understanding of dental healthcare professionals on the epidemiology, transmission, and prevention of HAIs. But a sociological research done among Osh State University's dentistry experts and students found gaps in knowledge and misunderstandings about infection safety. Although most dentists and residents (79.4±2.5%) and students (65.9±3.22%) showed correct knowledge of the main transmission routes of HIV and viral hepatitis B and C, a noteworthy proportion (21.6±2.6%) were unable to fairly evaluate infection risk. Unbelievably, 65.7±3.22% of students were ignorant about the availability of the hepatitis B vaccination and 42.5±3.44% lacked information on avoiding viral hepatitis B and C.

Though infection control training is very important, only 14.5±2.2% of the physicians polled had completed certified infection control courses over the preceding three years. Moreover, 49.2±3.1% said they have received instructions or training on infection control strategies at their places of employment. The main sources of knowledge on HAIs in dentistry differed among the respondents, which emphasizes the necessity of consistent teaching programs and strengthening of infection control practices within dental clinics.

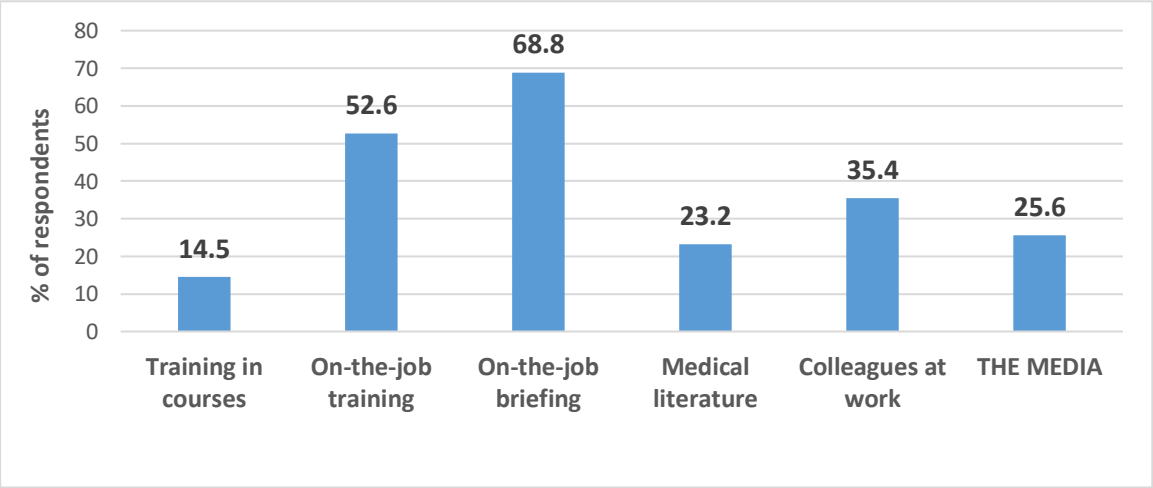


Figure 1.Answers to the question"Main sources of information on the epidemiology of GKI in dentistry"(in%)

Figure 1 shows that for most respondents, occupational-based training—including seminars and instructional courses—is the main source of knowledge about healthcare-associated infections (HAIs). But only a small percentage of workers (14.5±2.2%) said they had attended specialist seminars for recognized training, therefore exposing a discrepancy in official infection control instruction.

3.5 Concern and Challenges

Important markers of infection safety in dentistry offices are the condition of the working surroundings and the suitability of the tools. Among the respondents dentists and residents, 42.5±3.0% evaluated their working

circumstances as "good," 52.5±3.1% said they were "satisfactory," and 5.0±1.4% said they were "unsatisfactory." The respondents pointed out some issues with workplace organization. While 44.5±3.1% expressed inadequate workspace as a main issue, 30.5±2.8% of respondents said their work organization was ineffective. Furthermore noted by 32.4±2.9% of respondents as insufficient illumination levels and 25.6±2.7% as a contributing cause to less than ideal working conditions was poor ventilation. These results highlight the importance of bettering the physical surroundings of dental offices in order to improve infection control strategies. Ensuring a safer and more effective dental office environment depends on addressing these problems via improved infrastructure, well-designed workflow, and focused infection control training courses [20].

The expert will answer the question " The level of equipment of adental organization with dental and sterilization equipment, tools and materials. We received the following responses (Figure 2).

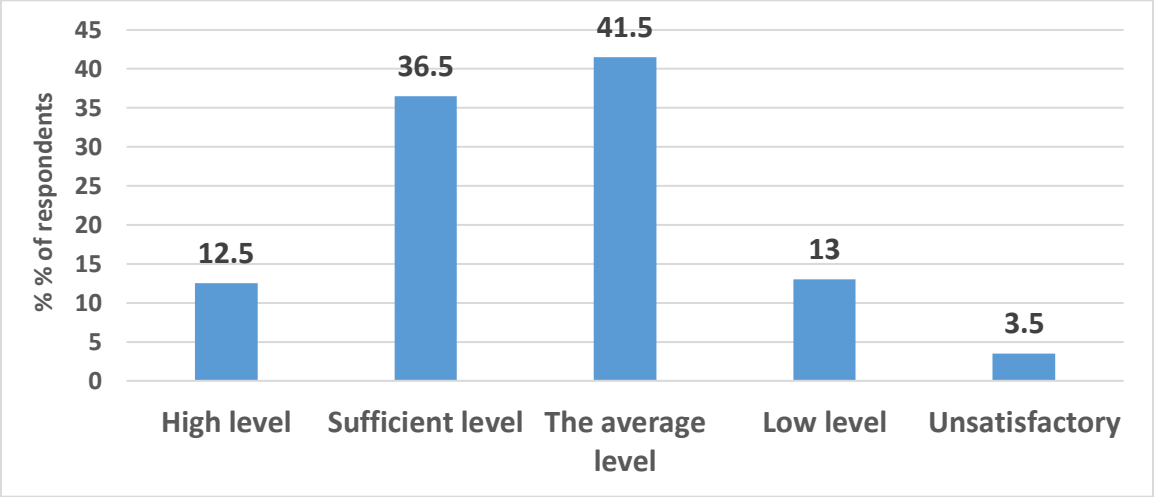


Figure 2 Assessment of the level of equipment of a dental organization (in %)

According to Figure 2, most of the respondents—78.0±2.6%—said their polyclinic's equipment satisfied minimum criteria. Some responders, however, voiced worries citing a "low level" or "unsatisfactory" equipment condition. Examining medical staff members' adherence to anti-epidemic policies during patient visits became the second step of the research. Preventing the spread of infectious disease pathogens to patients and medical staff depends critically on ensuring the safety of medical operations, the use of personal protective equipment (PPE), and hand hygiene practices. Figure 3 shows the results of the poll of dental institution workers about these features.

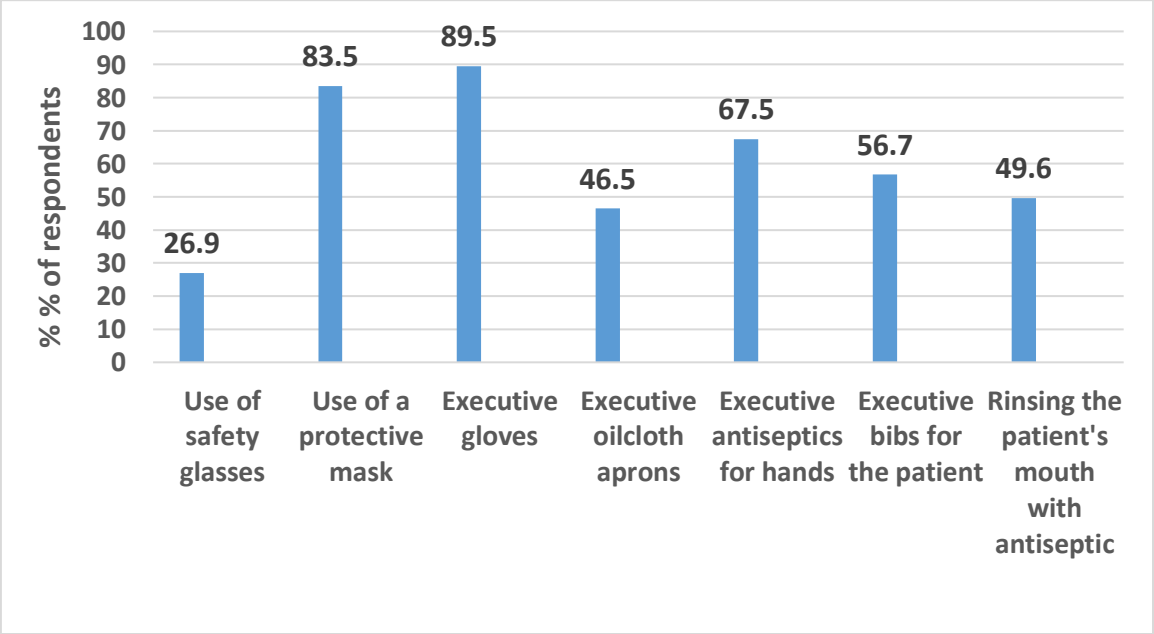


Figure 3. Results of a survey on the implementation of measures for the safety of medical manipulations in dental clinics.

As Figure 3 shows, although 16.5±2.3% of patients do not regularly utilize disposable masks while getting dental treatment, 83.5±2.3% of patients do. Furthermore, more than half of the respondents—73.1±2.7%—said they did not use protective goggles when providing patient treatment. Lack of availability, forgetfulness, or difficulty wearing masks or glasses are among the claimed causes. For the usage of aprons, antiseptics, and mouthwashes, a similar trend was seen. The poll results also showed that dental offices often neglect to follow hygienic and epidemiological guidelines, therefore violating infectious safety norms during patient contacts. Especially, 69.5±2.9% of clinicians never ask if their patients have viral hepatitis B or C. Furthermore, 34.5±3.0% of the physicians asked said they had varied degrees of skin damage or needle-stick events while working. These results show the great risk of hemocontact infections (HCIs) for patients and staff members of dental companies. Among the contributing causes are declining socioeconomic circumstances, inadequate financing for state medical facilities, and a dearth of contemporary sterilizing tools [21]. Furthermore, the limited resources and materials make it difficult to follow sanitary and anti-epidemic standards; meanwhile, poor understanding among medical staff on infection control aggravates the problem [22]. Thus, strict infection safety measures in dental offices must be followed and enforced in order to protect patients as well as medical staff [23].

5. CONCLUSION

the Osh region of the Kyrgyz Republic, the epidemiological represent concerns infectious diseases with a hemocontact transmission mechanism, including viral hepatitis B and C, as well as HIV, which continues to be a serious threat to public health. With numbers of 19.7±1.4 and 10.7±1.4 respectively, the data of 2020 to 2024 shows that the rates of hepatitis B (HBV) and C (HCV) among healthcare professionals have remained shockingly constant. These numbers show an ongoing risk with no improvement in lowering the transmission rates during this time. Furthermore under close consideration is the dentistry sector as regular breaches of approved hygienic and epidemiological guidelines occurs. Particularly in patient care, dental groups can neglect to follow infection safety procedures, thus increasing the danger of cross-contamination and hemocontact infection transmission, so compromising both medical staff and patients. Implementing a multifarious strategy to infection control is quite essential to meet these difficulties. This includes enhancing monitoring to pinpoint possible infection sources, funding contemporary sterilization and disinfection technology to guarantee the safety of medical equipment, and guaranteeing rigorous adherence to sanitary and anti-epidemic policies in dental offices. Furthermore important procedures to reduce the risk of transmission and protect the community as well as the healthcare personnel are improving control of infections and strengthening of the execution of preventive actions. These all-encompassing policies will help to lower the prevalence of certain avoidable diseases and enhance the general state of public health in the area.

DECLARATIONS

Ethical statement
This study was performed in line with the principles of the Declaration of Helsinki.

Consent statement
Due to the retrospective nature of the study, informed consent was waived

Data availability statement
Data will be made available on request.

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This research did not receive any specific funding.

Declaration of competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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RESEARCH ARTICLE

PREVALENCE OF DENTAL CARIES AMONG SCHOOLCHILDREN OF CHON ALAI DISTRICT OF THE KYRGYZ REPUBLIC

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ABSTRACT

Background: The worldwide decrease in dental caries prevalence during recent decades has not included sufficient research on rural high-altitude populations from Central Asia. The burden of dental caries among schoolchildren in geographically isolated mountainous regions of Kyrgyzstan remains poorly documented because socioeconomic constraints and dietary habits and healthcare access disparities may increase disease risk. This research examines the dental caries epidemiological patterns together with their changeable risk factors among schoolchildren in the unexplored rural highlands of southern Kyrgyzstan.

Research methods: The study included 413 students from Myrzakulov Secondary School located in Chon Alai District of Osh Region. The researchers obtained demographic information along with ethnic background and behavioral data through anonymous structured questionnaires that assessed oral health knowledge and attitudes and practices. The analysis of caries prevalence (percentage affected) and intensity (dft/DMFT indices) used descriptive statistics and logistic regression methods.

Results: The prevalence of caries in deciduous and permanent dentition was 94.2% and 95.5%, respectively. The prevalence of caries in permanent teeth was 77.7% among children aged 8–17 years. The dft score of 6-year-olds was 6.25 ± 1.4 and the DMFT index of 12-year-olds was 4.81 ± 0.79, which indicates a subcompensated caries intensity level. The proportion of restored teeth (f-component) was found to be low in all age groups.

Conclusion: The dental caries prevalence among rural Kyrgyz highland schoolchildren reaches concerning levels in their primary and permanent teeth. The results demonstrate the need to strengthen school-based preventive programs and implement oral health education to reduce sugar consumption and extend restorative dental services to underprivileged areas.

Keywords*:* Dental caries, epidemiology, disease prevalence, caries intensity, rural highland populations, Kyrgyzstan

1. INTRODUCTION

Dental caries stands as one of the leading chronic diseases worldwide which disproportionately affects children who live in low-resource areas and geographically isolated communities. The World Health Organization (WHO) reports that untreated dental caries in permanent teeth affects 2.3 billion people worldwide and developing region children suffer most because they

lack access to preventive and restorative dental care[1]. The caries prevalence in urban and high-income settings has decreased due to oral health education and fluoridation programs and dietary guidelines but rural and mountainous populations in Central Asia still face systemic barriers to achieve oral health equity[2]. The landlocked country of Kyrgyzstan demonstrates the difficulties that its rugged terrain and high-altitude settlements create. The population of Kyrgyzstan exceeds 60% in rural areas where socioeconomic inequalities combine with insufficient healthcare facilities and carbohydrate-based diets to increase dental disease risk. Rural communities including those in Chon Alai district of Osh region receive inadequate attention from the country's oral health policies because they focus primarily on urban areas[3]. Most existing epidemiological research about dental caries in Kyrgyzstan studies urban pediatric populations while ignoring the distinct risk factors of children living in remote high-altitude areas. The current research gap requires studies that focus on specific contexts to develop effective intervention strategies[4].

The widespread dental caries problem among schoolchildren in Kyrgyzstan's rural highlands demonstrates an important public health issue which combines socioeconomic factors with environmental conditions and behavioral elements[5]. The regions show concerning high caries rates but researchers have not gathered complete information about disease severity and risk factors and preventive practices. The people of Chon Alai rural area face multiple barriers to dental care because they lack fluoridated water supply and have few dental facilities and traditional eating habits that include frequent consumption of sweetened tea and dried fruits along with carbonated beverages[6]. The combination of low parental understanding about oral hygiene and limited financial resources prevents families from obtaining prompt dental care. The lack of school-based oral health programs enables the cycle to continue which puts children at increased risk of experiencing pain and infection and missing school[7]. This research investigates caries prevalence together with intensity and modifiable risk factors within a high-altitude rural population[8]. The research findings have global implications because mountainous regions across the Andes and Himalayas and other parts of the world face identical obstacles including geographical barriers and cultural eating habits and inconsistent healthcare systems. The study reveals local determinants through its findings which establish a framework to analyze caries epidemiology in comparable settings and promote targeted solutions to reduce oral health disparities[9,10].

The research adds fresh knowledge to worldwide oral health research by studying schoolchildren in remote high-altitude communities of Kyrgyzstan.

The majority of Central Asian research has analyzed urban populations or used outdated national surveys which fail to properly represent remote mountainous areas. The study provides a comprehensive epidemiological dental caries profile of this unique population by using WHO-standardized diagnostic criteria together with structured questionnaires that collect demographic and behavioral and socioeconomic data. The research will establish caries patterns by age group for both primary and permanent teeth while assessing the effects of diet and oral hygiene habits and determining care access challenges to develop evidence-based interventions. The study introduces the "subcompensated" caries intensity classification to describe this population's disease progression which shows moderate severity without adequate restorative treatment to guide clinical decision-making in limited resource settings. The results demonstrate the critical requirement for school-based preventive programs and policy reforms to increase dental workforce distribution in rural areas through their evidence of low filled teeth (f-component) and elevated untreated decay rates. The research outcomes will guide Kyrgyzstan's national oral health strategy development while providing reference material for mountainous regions dealing with similar challenges to establish cross-regional partnerships for addressing rural pediatric oral health neglect.

2. MATERIAL AND METHODS

2.1 Study Design

The research took place as a cross-sectional epidemiological study during November 2022 at Myrzakulov Secondary School located in Daroot Korgon village of Chon Alai district in Osh region within the Kyrgyz Republic. The village located at a high-altitude mountainous location which reaches 2,468 meters above sea level and contains 4,726 residents according to the 2009 census. The research evaluated dental caries prevalence and intensity and risk factors among 6-17 year-old schoolchildren while following national dental prevention programs and ethical standards from the Kyrgyz Ministry of Health and the Helsinki Declaration [11].

2.2 Study Setting and Population

The research included all students who enrolled at Myrzakulov Secondary School with a total number of 413 participants. The study excluded students who were absent from school because of illness during the three-day examination period (n = 27). The research participants included students from various ethnic

groups mainly consisting of Kyrgyz and Uzbek students who matched the population demographics of Chon Alai district.

The research included students whose parents provided written consent and who gave their own written agreement to participate without any age or gender limitations.

2.3 Materials and Data Sources

2.3.1 WHO Diagnostic Criteria: The dental examinations used standardized WHO protocols (2013) to evaluate caries prevalence and intensity. The evaluation of primary and permanent dentition used decayed, filled teeth (dft) and Decayed, Missing, Filled Teeth (DMFT) indices. The assessment included records of caries complications and teeth that required extraction because of caries and teeth that received sealing[12].

2.3.2 Demographic and Behavioral Data: A structured anonymous questionnaire captured age, gender, ethnicity, parental education, oral hygiene practices (e.g., toothbrush frequency, fluoride use), dietary habits (sugar consumption, carbonated drinks), tobacco use initiation, and dental visit frequency.

2.4 Data Collection Procedures

2.4.1 Clinical Examination: Two trained dentists conducted visual-tactile examinations under natural light conditions by using sterilized probes and mirrors. The examiners received calibration to achieve inter-rater reliability (kappa = 0.85). The WHO oral health assessment forms were used to document the findings.

2.4.2 Questionnaire Administration: The distribution of self-administered questionnaires by teachers occurred during school hours while maintaining anonymity. The instructions were delivered in both Kyrgyz and Uzbek languages to support students with different linguistic backgrounds.

2.5 Caries Assessment Criteri

2.5.1 Primary Dentition (dft): Calculated as the sum of decayed (d), filled (f), and teeth extracted due to caries (t) in children ≤12 years.

2.5.2 Permanent Dentition (DMFT): Sum of decayed (D), missing (M), and filled (F) teeth in children ≥6 years. Caries intensity was classified as *subcompensated* (DMFT 3.5–6.4) based on WHO thresholds.

2.6 Statistical Analysis

Data were analyzed using IBM SPSS Statistics v.19. Descriptive statistics—means, standard deviations, percentages—summed caries frequency (dft/DMFT) and behavioral variables. While t-tests examined variations in caries severity, chi-square tests evaluated categorical variables—e.g., caries rates by age group. High DMFT scores were linked, according to multivariate logistic regression, to risk variables including sugar intake, irregular brushing. Set significance at *p* 0.05.

2.7 Ethical Statement

The Institutional Review Boards of Osh State University and I.K. Akhunbaev Kyrgyz State Medical Academy approved the study through Protocol No. 14 on June 3, 2021. The researchers obtained written consent from parents/guardians and student assent from participants. The research maintained complete data anonymity while following both the Helsinki Declaration and Kyrgyz national ethical guidelines for human subjects research.

3. RESULTS

In all, 413 students between the ages of 6 and 17 years old who were enrolled in grades 1-11 were included in the research. The gender and age distribution of the participants is shown in Table 1, which provides information about individually.

Table 1. Characteristics of study participants (n-413).

N	Age	Boy	Girl	Total
	6	8	11	20
	7	17	22	39
	8	15	17	32
	9	27	27	54
	10	24	19	43
	11	26	23	49
	12	14	23	37
	13	14	14	28
	14	17	22	39
	15	10	16	26
	16	8	9	17
	17	4	9	13
Σ		202	211	413

The occurrence of caries in primary teeth among children aged 6 to 7 years was found to be 94.2%. The occurrence of permanent teeth caries among children aged 11-12 years was found to be 95.5%. The occurrence of caries in permanent teeth among children aged 8 to 17 years stands at 77.7%. Table 2 illustrates the occurrence and severity of dental caries in schoolchildren categorized by age.

The occurrence of caries in primary teeth among children aged 6 to 7 years was found to be 94.2%. The occurrence of permanent teeth caries among children aged 11-12 years was found to be 95.5%. The occurrence of caries in permanent teeth among children aged 8 to 17 years stands at 77.7%. Table 2 illustrates the occurrence and severity of dental caries in schoolchildren categorized by age.

Table 2. Prevalence and intensity of dental caries among schoolchildren according to age (n- 413).

Age	*n*	*D*	*F*	*M*	*DMFT*	*d*	*f*	*df*
6	20	0,35±0,08	0	0	0,35±0,08	6,25±1,4	0	6,25±1,4
7	39	0,28±0,04	0	0	0,28±0,04	6,67±1,07	0,08±0,01	6,75±1,08
8	32	0,41±0,07	0,06±0,01	0	0,47±0,08	5±0,88	0,13±0,02	5,13±0,9
9	54	1, 20±0,16	0,06±0,01	0	1,26±0,17	3,63±0,49	0,07±0,01	3, 70±0,5
10	43	2,09±0,32	0,19±0,03	0,05±0,01	2,33±0,36	1,67±0,25	0,02±0,03	1,69±0,26
11	49	3,69±0,53	0,18±0,03	0,06±0,01	3,93±0,56	0,16±0,02	0	0,16±0,02
12	37	4,11±0,69	0,54±0,09	0,16±0,03	4,81±0,79	0,38±0,06	0	0,38±0,06
13	28	2,5±0,47	0,5±0,09	0,04±0,01	3,04±0,57	0,04±0,01	0	0,04±0,01
14	39	3, 10±0,49	0,36±0,06	0	3,46±0,55	0,08±0,01	0	0,08±0,01
15	26	2,96±0,47	0,81±0,13	0,35±0,06	4,12±0,66	0,04±0,01	0	0,04±0,01
16	17	3,06±0,74	0,41±0,1	0,24±0,06	3,71±0,9	0	0	0
17	13	2,92±0,8	1,38±0,38	0,31±0,09	4,61±1,28	0	0	0
	413	2,23±0,11	0,31±0,02	0,08±0,03	2,62±0,13	2,12±0,13	0,03±0,01	2,15±0,16

4. DISCUSSION

By ethnic composition, the children mostly belonged to the Kyrgyz population. There are isolated cases where people of Tajik ethnicity reside in the area and the neighboring mountainous regions. We have not found any difference in the prevalence and intensity of dental caries and oral diseases based on ethnicity. In Table 2, it is seen that the average intensity of caries of deciduous teeth in children aged 6 years was 6.25 ± 1.4, and in children aged 7 years, it was 6.75 ± 1.08. The intensity index of caries of permanent teeth (DMFT) in 12-year-old children was 4.81±0.79. Based on the caries intensity classification created for the Kyrgyz Republic by Prof. G. Cholokova in 2000, the level of dental caries (in both baby and adult teeth) is considered sub-compensated. The low percentage of the index of *f* – filled teeth is noteworthy. For 6-year-olds, it amounted to 1.2% of the total index. 11.2% of the 12-year-old students' entire index was made up of sealed teeth. In general, for all ages of schoolchildren, only 11.8% of the total DMFT index consisted of sealed teeth. Such low indicators show the insufficiency of the ongoing prophylactic and rehabilitation work among schoolchildren[13].

The situation is also evidenced by the results of an anonymous survey conducted among 225 schoolchildren, mainly middle and older age groups (130 of them boys and 151 girls). 63.3% of respondents rated the condition of their teeth as "excellent," "good," or "satisfactory." The condition of the gums was also assessed by 52%. Additionally, 13% of schoolchildren found it “difficult to answer” the first question, and 9.6% found it difficult to answer the second question. To the question: "Have you visited a dentist in the last 12 months?" 59% answered yes often, sometimes, or rarely. 28% of respondents have never visited a dentist, and 13% found it difficult to answer this question. To the same question, asked in a different" way—"How often have you visited the dentist in the last 12 months?"—the" answer was also identical (59%). 1-2 times-(36%), 3 or more times-23%. 20% of respondents answered that they had not visited a dentist, and 14% found it difficult to answer this question. In the rest of the answers, the students visited the dentist only a few times. For 49% of respondents, the reason for visiting the dentist was either pain (31%) or dental treatment (18%). Only 18% of respondents applied to a dentist to examine the condition of their teeth and oral cavity. 26% found it difficult to answer this question. Of the total number of respondents, 89 (32%) brush their teeth two or more times a day and 107 (38%) once a day. 74 (26%) rarely brush their teeth, of which 32 (11.4%) do

so several times a month, 15 (5%) once a week, and 27 (9.6%) several times a week[14]. Of the oral hygiene products, 90% of respondents use a toothbrush, 19% use wooden toothpicks, 10% use plastic toothpicks, 11% use miswak, and 7.5% use dental floss.93% of respondents use toothpaste; 11% use toothpaste with fluoride content, and 26% use toothpaste without fluoride content. 21% of respondents found it "difficult to answer" this question. The next group of questions concerned the subjective assessment of the condition of one's own teeth and problems with them. According to the answers received, 27% (76) of respondents are not satisfied with the condition of their teeth, and 22% found it difficult to answer this question. 13%—try not to smile because of the appearance of their teeth. 12% found it difficult to answer. 8% answered that the children laugh at the sight of my teeth, and 19% found it difficult to answer. 17% missed lessons due to toothache, and 14% found it difficult to answer. 12% replied that they had difficulty biting off food, and 8% found it difficult to answer. 15% have difficulty chewing food, and 9% found it "difficult to answer."

Some questions concerned the dietary preferences of schoolchildren and determined their indirect effect on the prevalence and intensity of diseases of tough dental tissues. According to the respondents' answers, it was determined that fresh fruits were consumed frequently-several times a day, daily, or several times a week—by 71% of the schoolchildren who answered, while 20% rarely consumed fresh fruits. Biscuits, cookies, and sweet pies are often consumed by 45%, and 60% often consume jam, while 17% consume it rarely. Further, chewing gum is often consumed by 48% and rarely by 26%. 48% consume sweets frequently, while 25% consume them rarely. Lemonade, cola, and other carbonated drinks are often consumed by 35% and rarely by 33%. Milk with sugar is often consumed by 38% and rarely by 32%. 52% often consume tea with sugar, while 20% rarely do so. National drinks Kumuz (from mare's milk) is often consumed by 12.5% and rarely by 60%. 8% often consume Zharma, the national drink, while 57% consume it rarely. National drinks-Kumuz and Zharma are low-alcohol, and their rare use, according to the results of the survey, suggests relatively reliable answers to questions, since they are not often consumed by school-age children.

The questionnaire included questions about the use of cigarettes, tobacco, and other smoking mixtures. According to the respondents' answers, 2% often use cigarettes (several times a day or a week), 16% rarely (1-2 times a month), and occasionally they have tried or respondents. Another type of tobacco-Nass chewing tobacco-is often used by 3%, rarely by 3%, and practically do not smoke-88% of the occasionally they have tried it or practically do not use it—80%. Regarding parental education, we received the following responses. The education of the father or guardian in 4% of cases is lower than secondary or primary, in 16% secondary, and in 32% higher education, institute, and university. In 3.5% of cases, the mother's education is lower than average or primary; in 13%, secondary; and in 42%, higher education, institute, and university. 41% of respondents could not determine the level of education of their father and mother.

This research was carried out for the very first time in Kyrgyzstan since it gained its independence. Studies that were quite similar to this one were carried out in the past, either in the valley and the mid-mountain levels of the republic, or in the towns of the republic. More than ninety percent of the land of the country is comprised of hilly regions. Based on the findings of our research, we discovered that the high-mountainous regions have a higher incidence of caries. In terms of dental caries intensity, it is somewhat equivalent to that of other locations[15]. As a result of the comparatively limited accessibility of high-altitude locations, relatively poor indicators of schoolchildren's knowledge of the detrimental effects of sugars, sugar-containing goods, and carbonated beverages on dental and oral health were identified[16]. An insufficient level of primary prevention of caries and diseases of hard tissues of teeth is indicated by the fact that schoolchildren are unable to determine the level of education of their parents (41 percent of respondents), or that the level of education of their parents is low (four percent of them have levels of education that are below average or primary, and thirteen to sixteen percent of them have secondary levels of education). Dental caries is a prevalent condition characterized by a moderate level of severity[17]. The inadequate implementation of preventive measures, coupled with the limited educational background of parents, can also be attributed to the climatic and geographical characteristics of Kyrgyzstan, particularly the deficiency of essential minerals such as calcium, fluorine, and iodine in the drinking water supply. The mountainous regions of Kyrgyzstan are characterized by a notable deficiency of fluorine and iodine, rendering them an endemic zone. In certain areas, access to clean drinking water is limited[17,18].

The data collected align well with findings from earlier research. Our investigation revealed a significant occurrence of dental caries. It is recognized that the occurrence of caries is not directly influenced by a country's economic development. However, the implementation of social hygiene initiatives and preventive strategies can contribute to stabilizing or

lowering the intensity of this condition. Our findings indicate that ethnicity did not have a significant impact on the index indicators related to the prevalence and intensity of dental diseases[19]. Our findings indicate that climatic and geographical living conditions, awareness of primary prevention measures, and the availability and regular implementation of dental preventive care have a significant influence. This locality is home to a regional hospital and a regional family medicine center. The Family Medicine Center comprises essential specialists and divisions within the medical service. There are dental offices available, but there are only two dentists present, excluding any specialists in stomatology. Dental X-ray equipment absence at both the district hospital and Family Medicine Center prevents healthcare providers from detecting non-visible tooth areas and surfaces including approximal and subgingival regions. The inability to diagnose caries on invisible tooth surfaces becomes a challenge because of the limited diagnostic capabilities. The remote mountainous regions of Kyrgyzstan face a major challenge because of their healthcare professional shortage. State insurance covers medical dental services with planned preventive work but the available number of dentists and stomatologists fails to meet patient needs[20].

It is essential to consider the educational background of parents; notably, 41% of children were unable to identify their parents' level of education. In 4% of instances, it was found that the educational attainment of the parents was classified as low and below the average standard. The educational level does not provide adequate understanding of the basic requirements for implementing preventive measures for children and school-aged individuals. The research did not include an examination of schoolteachers' knowledge and awareness regarding the skills necessary for conducting health lessons, particularly in the area of oral health[12,22]. The research indicated that schoolchildren exhibited a limited understanding of dental disease prevention, particularly regarding caries. Kyrgyzstan has initiated a program aimed at preventing caries and other significant dental diseases. However, it has not yet been adopted at the state level. We believe it is essential for the program to encompass the enhancement of knowledge among parents and educators, particularly those teaching at the primary level, regarding the prevention of significant dental diseases[23].

5. Future recommendation to combat the challenges:

5.1 *Integrate School-Based Oral Health Education into National Curricula:* Create educational modules about oral health for students at primary and secondary school levels which cover hygiene practices and dietary guidelines and caries prevention. The training of teachers especially in rural areas to deliver these modules will enable children to become behavioral change agents for their families. The approach supports WHO school-based health promotion guidelines while addressing the study's findings about limited awareness[24].

5.2 *Implement Targeted Fluoridation Programs in Mountainous Regions:* The endemic fluoride deficiency and mineral-poor water sources require the implementation of cost-effective fluoridation strategies through salt fluoridation or school-based fluoride varnish applications. The caries incidence in high-altitude districts such as Chon Alai could decrease by 20–40% through similar interventions that have been successful in LMICs[25.]

5.3 *Strengthen Rural Dental Workforce Capacity:* The critical shortage of dental professionals in mountainous areas should be addressed by providing incentives for dentists to practice in rural areas through scholarships, housing allowances, or loan forgiveness. Furthermore, train mid-level dental therapists to perform basic preventive and restorative care, as recommended by the FDI World Dental Federation for underserved regions[26].

5.4 *Deploy Mobile Dental Units with Portable Diagnostic Tools:* Mobile clinics should receive compact radiographic devices (handheld X-ray units) and tele-dentistry capabilities to enhance early caries detection in remote villages. This would bypass the lack of fixed infrastructure and enable timely management of approximal and subgingival lesions, reducing complications[27.]

5.5 *Launch Parental Literacy Campaigns on Preventive Care:* Community leaders and local media should work together to spread educational materials about sugar reduction and fluoride use and routine dental visits which are culturally appropriate. The educational materials should use low-literacy formats such as visual posters and radio broadcasts to reach parents who have limited formal education since they make up 17% of the study population. The distribution of affordable hygiene kits containing toothbrushes and fluoride toothpaste by NGOs would help strengthen these messages[28].

6. CONCLUSION

The study reveals a significant oral health problem impacting high-mountain regions of Kyrgyzstan kids as restorative dental treatment is still inadequate and dental caries rates are very high. Treatment limitations have caused the caries intensity categorization of subcompensated to show that the disease has progressed to moderate to severe degrees. The

extremely low rates of filled teeth across all age groups demonstrate major shortcomings in dental service delivery and access that exceed global standards.

The high prevalence of dental caries in this populationstems from endemic mineral deficiencies and excessive consumption of cariogenic foods and poor oral health literacy among children and their caregivers. The research demonstrates how isolated populations face increased vulnerability because environmental conditions and socioeconomic factors and infrastructural limitations work together to worsen oral health problems. A comprehensive public health strategy needs to be established to tackle these challenges. The priorities should include school-based preventive programs combined with fluoridation initiatives and workforce task-shifting models and dietary regulations and culturally appropriate health education campaigns. The treatments must especially adapt for the unique socioecological setting of high-altitude areas. Resolving disease-causing elements at the population level calls for the harmonization of national oral health campaigns with global health criteria and sustainable development objectives. Future research needs to assess the effectiveness and scalability of proposed interventions to develop evidence-based policies that enhance outcomes for similar underserved populations.

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Ethics approval and consent to participate: All procedures performed in the present study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the declaration of Helsinki 1975, which was revised in 2000. Approval to conduct the study was obtained from the Institutional Ethics Board of the Osh State University and IK Akhunbaev Kyrgyz State Medical Academy (N14, June 03, 2021).

Competing interests: The authors declare that they have no competing interests.

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REVIEW ARTICLE

MARINE SEAWEED POLYSACCHARIDES AS HERBAL THERAPEUTICS IN ORAL CANCER AND ORAL HEALTH MANAGEMENT – A NARRATIVE REVIEW

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ABSTRACT

Background: Herbal remedies are gaining recognition in oral and dental healthcare for their antimicrobial, anti inflammatory, and therapeutic benefits. Marine seaweed polysaccharides (MSPs) including alginate, carrageenan fucoidan, and agar are derived from brown, red, and green algae. These natural compounds exhibit diverse biological activities such as antioxidant, immunomodulatory, and anticancer effects, making them promising candidates for the integrative management of oral squamous cell carcinoma (OSCC), a prevalent and aggressive oral malignancy.

Purpose: This review explores the therapeutic potential of MSPs in OSCC and broader oral health applications. It highlights their mechanisms of action, evaluates preclinical and early clinical evidence, and discusses their potential as adjuncts in dental and oncological care.

Methods: A systematic literature search was conducted using PubMed, Scopus, and Web of Science databases for studies published in the past five years. Keywords included "marine polysaccharides," "seaweed," "oral cancer," "OSCC," "oral health," and "herbal medicine." Relevant *in vitro*, in vivo, and clinical studies were included. The review was conducted in accordance with the PRISMA guidelines. Figure 1 presents the PRISMA flow diagram outlining the identification, screening, eligibility, and inclusion of studies considered for this review.

Results: MSPs demonstrate anticancer effects through induction of apoptosis, inhibition of proliferation angiogenesis, and metastasis, and modulation of immune responses. Their antioxidant and anti-inflammatory properties also support roles in managing periodontitis and gingivitis. Favorable bioavailability and safety profiles enhance their applicability in drug delivery and oral therapeutics.

Conclusions: Marine seaweed polysaccharides offer a promising herbal approach for OSCC and oral health disorders. Further clinical and mechanistic studies are essential to validate their efficacy and support their inclusion in evidence-based dental practice.

Keywords: Anticancer; Antioxidant; Cancer; Immunomodulatory; Oral; Marine-Seaweed

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1. INTRODUCTION

Oral squamous cell carcinoma (OSCC) poses a significant global health concern, accounting for the majority of oral cancers and approximately 3% of all malignancies worldwide.[1,2] It is characterized by late-stage diagnosis, aggressive clinical progression, and limited therapeutic responsiveness, often leading to poor prognosis and reduced quality of life for affected individuals.[3,4] Conventional treatment options such as surgery, chemotherapy, and radiotherapy are associated with substantial side effects and frequently yield suboptimal outcomes, particularly in advanced or recurrent cases.[1,5] These limitations underscore the urgent need for novel, safer, and more effective therapeutic strategies for OSCC management. Marine organisms, particularly seaweeds, have gained attention as promising sources of bioactive compounds with pharmaceutical relevance in cancer therapy. Classified based on pigmentation into brown (Phaeophyceae), red (Rhodophyceae), and green (Chlorophyceae) algae, seaweeds produce structurally diverse polysaccharides with a wide array of biological activities. Notable compounds include alginate, carrageenan, fucoidan, and agar, each exhibiting potential applications in drug delivery, immunomodulation, and cytotoxicity against cancer cells.[5,6] For example, alginate from brown algae is well known for its biocompatibility and mucoadhesive properties, making it suitable for use in oral drug delivery systems.[8] Carrageenan, derived from red algae, possesses immunomodulatory and antioxidant activities, while fucoidan has shown the ability to induce apoptosis and inhibit angiogenesis in various cancer models.[9]

Recent preclinical studies have further demonstrated the anticancer potential of these marine-derived polysaccharides in OSCC models. Fucoidan, in particular, exhibits cytotoxic effects by inhibiting cell proliferation and inducing apoptosis through modulation of key oncogenic signaling pathways.[9,10] Additionally, these polysaccharides have shown the capacity to enhance the efficacy of chemotherapeutic agents by minimizing systemic toxicity and improving tumor responses.[5,11] Such synergistic effects may revolutionize current OSCC treatment paradigms, especially for patients unresponsive to standard therapies. The integration of marine polysaccharides into existing treatment regimens holds promise for improving outcomes in oral cancer management.

Several studies have reported that these compounds can act as adjuncts to conventional therapies, reducing adverse effects while enhancing therapeutic efficacy.[5,12,13,14] Continued research into their mechanisms of action is essential to facilitate clinical translation and therapeutic optimization. With their multifaceted bioactivity and low toxicity profiles, Marine seaweed polysaccharides offer a compelling opportunity to advance evidence-based, herbal-inspired approaches to oral cancer treatment. Their incorporation into modern integrative oncology may ultimately shift the trajectory of care for millions of patients worldwide.

This review explores the therapeutic potential of MSPs in OSCC and broader oral health applications. It highlights their mechanisms of action, evaluates preclinical and early clinical evidence, and discusses their potential as adjuncts in dental and oncological care.

2. METHODS

A systematic literature search was conducted using PubMed, Scopus, and Web of Science databases for studies published in the past five years. Keywords included “Marine Polysaccharides,” “Seaweed,” “Oral Cancer,” “OSCC,” “Oral Health,” and “Herbal medicine.” Relevant *in vitro*, *in vivo*, and clinical studies were included. The review was conducted in accordance with the PRISMA guidelines. Figure 1 presents the PRISMA flow diagram outlining the identification, screening, eligibility, and inclusion of studies considered for this review.

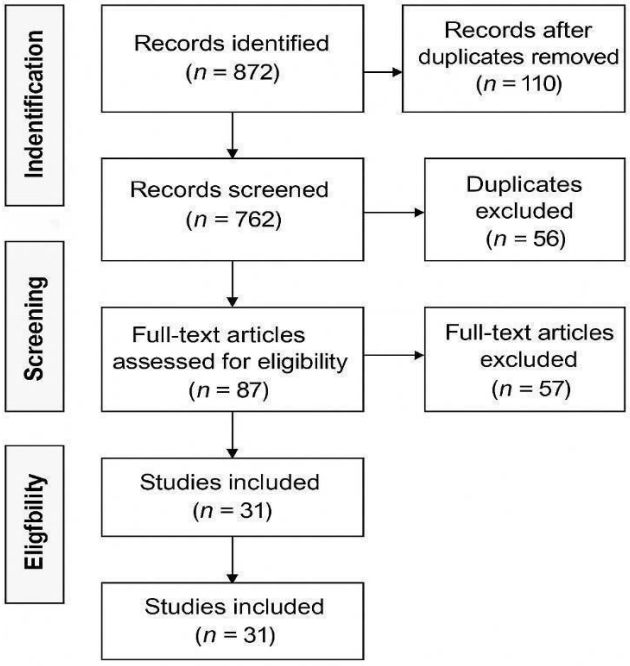


Figure 1. PRISMA Flow Diagram of the study

2. Mechanisms of Action in Oral Cancer Treatment

The development and progression of oral squamous cell carcinoma (OSCC) are governed by complex molecular mechanisms, many of which can be modulated by marine-derived herbal compounds such as fucoidan. As a sulfated polysaccharide extracted from brown seaweeds, fucoidan has demonstrated a range of anticancer activities, including apoptosis induction, inhibition of proliferation, anti-angiogenesis, and suppression of metastasis. These multifaceted actions make it a compelling candidate for integrative oral cancer therapy.

2.1 Inductionof Apoptosis

Apoptosis, or programmed cell death, is a vital mechanism for eliminating damaged or malignant cells. Fucoidan has been shown to activate both the extrinsic and intrinsic apoptotic pathways in OSCC cells.[15] It initiates apoptosis by activating caspase-8 (extrinsic) and caspase-9 (intrinsic), leading to the downstream activation of executioner caspase-3. Fucoidan also upregulates pro-apoptotic proteins such as Bax and downregulates anti-apoptotic Bcl-2, thereby increasing mitochondrial membrane permeability and promoting the release of cytochrome-C, a pivotal step in the intrinsic apoptosis pathway.[16,17]

2.2 Inhibition of Survival Signaling Pathways

In OSCC and many other cancers, the phosphatidylinositol 3-kinase (PI3K)/Akt pathway is frequently activated, contributing to uncontrolled cell survival and resistance to therapy. Fucoidan effectively inhibits the phosphorylation of Akt, leading to the downregulation of downstream survival signals.[18] This dual action promoting apoptosis while blocking survival pathways enhances its therapeutic potential.

2.3 Suppression of Cell Proliferation and Cell Cycle Arrest

Fucoidan also impedes cellular proliferation by inducing cell cycle arrest. It inhibits the mammalian target of rapamycin (mTOR) and mitogen-activated protein kinase (MAPK) pathways, reducing the expression of transcription factors such as c-Fos and c-Jun. This suppression leads to the downregulation of cyclin-dependent kinases (CDKs) and halts cell cycle progression at the G1/S checkpoint.[17,19]

2.4 Anti-Angiogenic Activity

Tumor angiogenesis is essential for cancer progression. Fucoidan has demonstrated the ability to inhibit angiogenesis by downregulating the expression of vascular endothelial growth factor (VEGF) and its receptor VEGFR at both transcriptional and translational levels. It also interferes with endothelial cell migration, tube formation, and survival by modulating the PI3K/Akt/mTOR signaling cascade.[20] Furthermore, fucoidan reduces the expression of hypoxia-inducible factor-1 alpha (HIF-1α), a major regulator of angiogenesis under hypoxic tumor conditions, contributing to reduced micro vessel density and impaired tumor blood supply.

2.5 Inhibition of Metastasis

Metastasis is a critical factor in the poor prognosis of OSCC. Fucoidan inhibits metastatic behavior by modulating the epithelial-to-mesenchymal transition (EMT). It upregulates epithelial markers such as E-cadherin while downregulating mesenchymal markers like N-cadherin and vimentin, thereby reducing cell invasiveness. Additionally, fucoidan suppresses matrix metalloproteinases (MMP-2 and MMP-9), enzymes responsible for extracellular matrix degradation, further hindering tumor invasion and migration.[18]

2.6 Modulation of the Tumor Microenvironment

The tumor microenvironment (TME) plays a central role in immune evasion and tumor progression. Fucoidan can reshape the TME by modulating immune and stromal cell behavior. It reduces the secretion of pro-inflammatory cytokines and inhibits nuclear factor-kappa B (NF-κB) signaling, thereby creating an environment less conducive to tumor growth and dissemination.[21]

2.7 Synergy with Conventional Therapies

One of fucoidan's most promising features is its ability to enhance the efficacy of traditional cancer treatments. Fucoidan-loaded nanoparticles have been developed for site-specific delivery of chemotherapeutic agents, reducing systemic toxicity. Moreover, fucoidan sensitizes OSCC cells to radiotherapy by increasing oxidative stress and impairing DNA repair mechanisms, enhancing the overall therapeutic response. [22] Fucoidan exhibits a robust and multi-targeted mode of action against oral squamous cell carcinoma, impacting key processes such as apoptosis, angiogenesis, metastasis, and immune regulation. Its ability to modulate several oncogenic pathways, combined with its synergy with existing treatments, makes it a promising marine-derived herbal agent in the integrative management of OSCC. Future studies should continue to investigate its molecular mechanisms, optimize delivery systems, and validate its clinical utility in large-scale trials.

3. Chemical Analysis of Marine Seaweed Polysaccharides

Marine seaweed polysaccharides namely alginate, carrageenan, fucoidan, and agar, possess structurally diverse and complex compositions that profoundly influence their physicochemical and biological properties (Figure 2, Table 1). A detailed understanding of their chemical structure is essential for optimizing their biomedical and therapeutic applications.

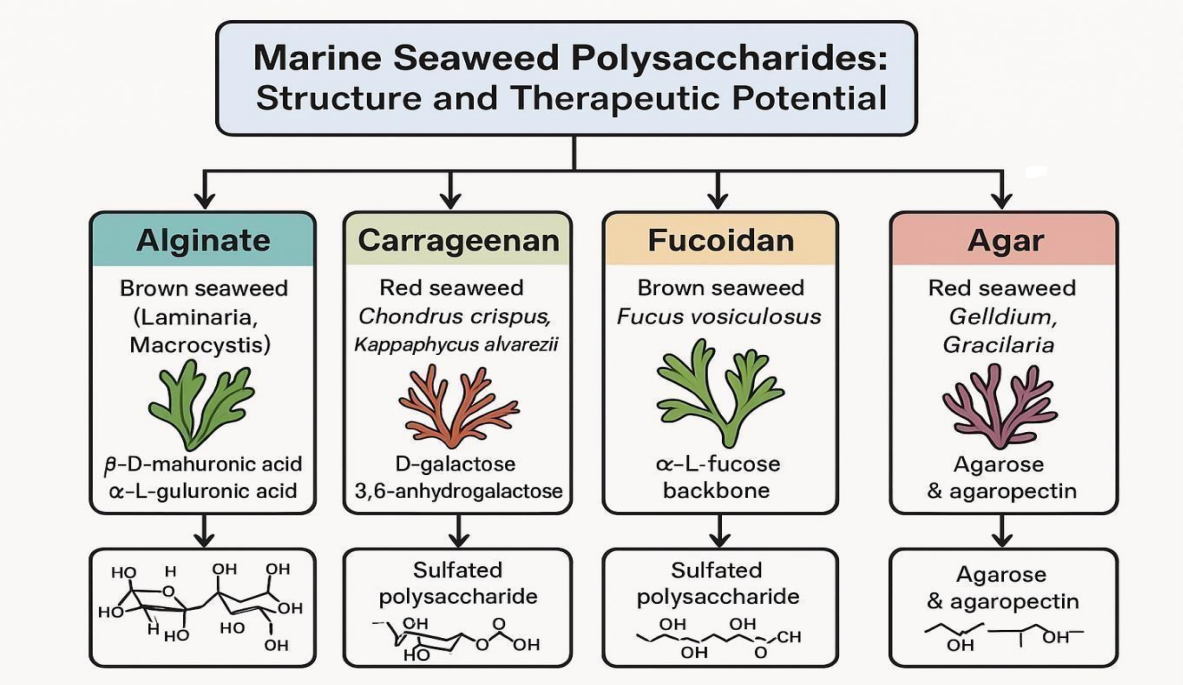


Figure 2. Graphical representation of major marine seaweed polysaccharides, highlighting their sources, structural components, and key characteristics.

Table 1. Chemical Characterization of Major Marine Seaweed Polysaccharides

Polysaccharide	Source (Seaweed Type)	Structural Features	Analytical Techniques	Key Properties / Applications	References
Alginate	Brown seaweeds (e.g., *Laminaria*, *Macrocystis*)	Alternating β-D-mannuronic acid and α-L-guluronic acid (1→4 linkages)	FTIR, NMR, HPLC	Gelation (esp. with G-blocks & divalent cations); viscosity control; biomedical & food industries	23,24
Carrageenan	Red seaweeds (*Chondrus crispus*, *Kappaphycus alvarezii*)	D-galactose and 3,6-anhydrogalactose with varying sulfation	FTIR, SEC, NMR	κ-, ι-, λ-types differ by sulfation and gel behavior; used in gelling, stabilizing, and pharmaceuticals	23,24,25
Fucoidan	Brownseaweeds (*Fucus vesiculosus*)	α-L-fucose backbone with sulfate groups and branching	MS, NMR, HPLC	Anticancer, immunomodulatory activity; structure-function depends on sulfation and branching	26,27,28
Agar	Red seaweeds (*Gelidium*, *Gracilaria*)	Agarose(linear galactan) + agaropectin	FTIR, NMR, DSC, Viscometry	Thermoreversible gel; high-purity agarose for microbiology and molecular biology	23,24,29
Functional Roles	Marine adaptation	Ion exchange, defense, and structure	–	Adaptation to salinity, pressure, predation; functional versatility	26,27
Emerging Applications	–	Structure-function dependent bioactivities	Advanced spectrometry, chromatography	Drug delivery, wound healing, cancer therapy, immunomodulation	26,30

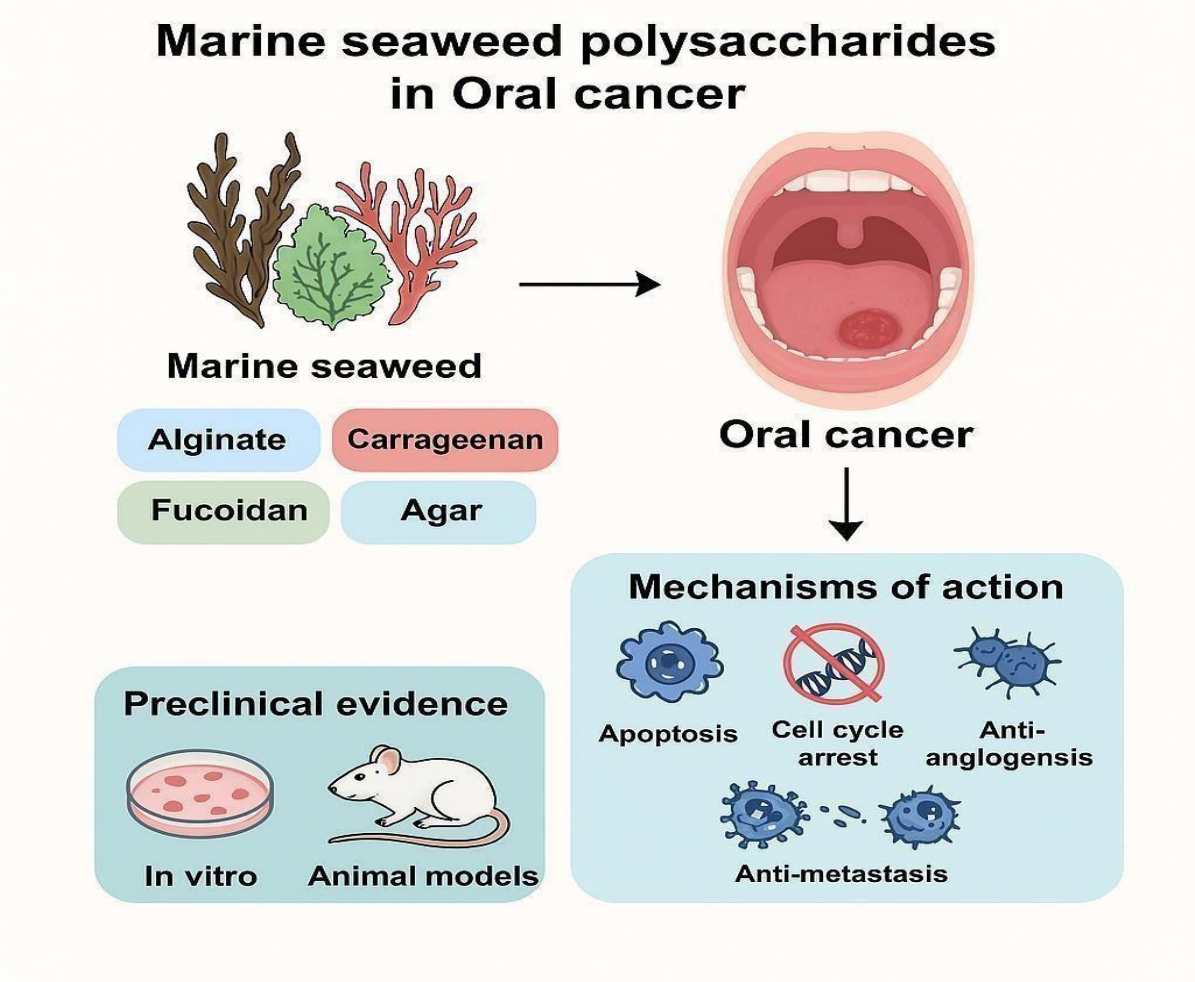


Figure 3. Graphical representation of mechanism of action Marine seaweed polysaccharides in oral cancer

3.1 Alginate

Alginate is a linear, anionic polysaccharide primarily extracted from brown seaweeds such as *Laminaria* and *Macrocystis*. It is composed of alternating blocks of β-D-mannuronic acid (M) and α-L-guluronic acid (G) linked via 1→4 glycosidic bonds. The ratio and sequential arrangement of these monomers determine alginate's gelation ability, viscosity, and bio functionality. A higher content of G-blocks enhances gel stability in the presence of divalent cations (e.g., Ca^{2+}), a property exploited in pharmaceutical and food-grade formulations.[23] Extraction typically involves alkaline treatment using sodium carbonate, followed by precipitation with ethanol or calcium chloride.

Analytical techniques such as Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), and high-performance liquid chromatography (HPLC) are employed to assess alginate's molecular weight distribution and functional group composition.[24]

3.2 Carrageenan

Carrageenan is a sulfated galactan derived from red seaweeds like *Chondrus crispus* and *Kappaphycus alvarezii*. It consists of alternating units of D-galactose and 3,6-anhydrogalactose, with varying degrees of sulfation and glycosidic linkages. Carrageenan is classified into three main types: kappa (κ), iota (ι), and lambda (λ), each with distinct gelling and rheological properties influenced by the presence of specific cations. κ-Carrageenan forms firm gels in the presence of potassium ions, while ι-carrageenan forms softer, more elastic gels with calcium ions.[23,25] Spectroscopic techniques (e.g., FTIR, NMR) and size-exclusion chromatography (SEC) are commonly used to analyze carrageenan's molecular composition and crystallinity.[24]

3.3 Fucoidan

Fucoidan is a sulfated polysaccharide predominantly found in brown algae such as *Fucus vesiculosus*. It features a backbone primarily composed of α-L-fucose units, often substituted with sulfate and other functional groups. Fucoidan's biological effects including anticancer, antiviral, anticoagulant, and immunomodulatory properties are largely dependent on its degree of sulfation, molecular weight, and branching patterns.[26] Its extraction typically involves aqueous or dilute acid extraction followed by alcohol precipitation. Advanced analytical techniques such as mass

spectrometry (MS) and NMR provide insights into its structural complexity and structure-activity relationships.[27,28,31]

3.4 Agar

Agar, derived from red seaweeds such as *Gelidium* and *Gracilaria*, is composed mainly of agarose and agaropectin. Agarose contributes to the strong gel-forming ability of agar, making it widely used in microbiology and pharmaceutical applications due to its thermal reversibility and biocompatibility.[24,29] The assessment of agar quality involves evaluating gel strength and melting/gelling temperatures through differential scanning calorimetry (DSC) and viscometric methods. Its chemical structure is further characterized by FTIR and NMR spectroscopy to determine glycosidic linkages and sulfate content.[23,24]

3.5 Structural Diversity and Biomedical Relevance

The structural diversity of marine seaweed polysaccharides reflects evolutionary adaptations to the marine environment and contributes to their wide range of biological activities. Alginate and carrageenan provide mechanical and ionic stability to the seaweed cell wall, while fucoidan and agar are associated with protective and ecological signaling roles.[2] Emerging advances in analytical technologies now allow deeper exploration into their structure–function relationships, offering pathways to understand the biochemical origins, branching patterns, and sulfation profiles of these macromolecules.[26] This knowledge is critical to designing therapeutic formulations that leverage their antioxidant, antimicrobial, anti-inflammatory, and anticancer activities. Ultimately, the chemical analysis of marine seaweed polysaccharides underpins their potential as multifunctional agents in modern herbal medicine and integrative oral healthcare. Continued research will enhance their standardization, formulation, and application in clinical and dental settings.[26,27,30]

4. Preclinical Evidence: Animal Models and *In vitro* Studies

Marine seaweed polysaccharides especially fucoidan, carrageenan, and alginate have been extensively studied in preclinical settings for their anticancer potential against oral squamous cell carcinoma (OSCC). Both *in vitro* cell line studies and in vivo animal models provide compelling evidence supporting their therapeutic efficacy, either as standalone agents or in combination with conventional therapies.

4.1 *In vitro* Studies

Experimental studies using human OSCC cell lines such as SCC-9, CAL-27, and HSC-3 have demonstrated the cytotoxic and anti-proliferative effects of marine polysaccharides. Fucoidan, derived from brown seaweeds, induces dose-dependent cytotoxicity, with reported IC_{50} values ranging from 50 to 200 µg/mL depending on the cell line and experimental parameters.[32,33] Mechanistic analyses indicate that fucoidan activates the mitochondrial apoptotic pathway, characterized by cytochrome c release, caspase-3 activation, and poly (ADP-ribose) polymerase (PARP) cleavage, confirming its role in programmed cell death.[32] Carrageenan, a sulfated polysaccharide from red algae, exerts its cytotoxicity through distinct mechanisms. It sensitizes OSCC cells to oxidative stress by depleting intracellular glutathione and increasing the production of reactive oxygen species (ROS), leading to DNA fragmentation and cell death.[33,34] Furthermore, carrageenan has been shown to inhibit the MAPK/ERK signaling cascade, a pathway integral to cancer cell survival and proliferation. While alginate is not directly cytotoxic, it plays a crucial role as a biocompatible drug delivery matrix. Alginate-based nanoparticles encapsulating chemotherapeutic agents such as cisplatin or doxorubicin have demonstrated improved intracellular drug accumulation and sustained release, resulting in enhanced anticancer efficacy compared to free drug formulations.[35] These findings underscore alginate's potential in optimizing chemotherapeutic outcomes in OSCC treatment.

4.2 In Vivo Studies

Animal model studies further support the therapeutic relevance of marine polysaccharides. In murine xenograft models of OSCC, daily administration of fucoidan (200 mg/kg) for four weeks led to a marked reduction in tumor volume. This was accompanied by decreased micro vessel density and downregulation of angiogenesis-related markers, including vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9), confirming its anti-angiogenic potential.[32] Similarly, dietary supplementation with carrageenan in a 4-nitroquinoline 1-oxide (4NQO)-induced oral carcinogenesis model significantly reduced the incidence of dysplastic lesions. Histopathological assessments revealed a decrease in inflammatory cell infiltration and epithelial abnormalities, indicating its chemo-preventive effects.[36]

4.3 Synergistic Potential with Conventional Therapies

Recent studies have explored the synergistic effects of marine polysaccharides when used alongside established cancer treatments. Fucoidan has been reported to enhance the efficacy of radiation therapy in OSCC models, with combination treatment resulting in improved tumor regression and prolonged survival.[32] Likewise, carrageenan has been shown to potentiate the effects of cisplatin, likely by amplifying ROS-mediated cytotoxicity and impairing cellular repair mechanisms.[34]

4.4 Safety and Toxicological Assessments

Marine polysaccharides generally exhibit a favorable safety profile. Repeated-dose toxicity studies in rodents have shown that fucoidan, administered at doses up to 500 mg/kg/day, does not result in significant changes in body weight, organ histology, or biochemical markers of toxicity.[33] Alginate and carrageenan are both classified as Generally Recognized as Safe (GRAS) by international food and drug regulatory agencies, supporting their use in biomedical applications.[37,38] However, care must be taken when using degraded or low-molecular-weight carrageenan, as some forms have been associated with pro-inflammatory responses depending on dosage and structural characteristics. Collectively, preclinical evidence strongly supports the therapeutic relevance of marine seaweed polysaccharides in OSCC treatment. Fucoidan and carrageenan demonstrate direct cytotoxic and immunomodulatory effects, while alginate enhances chemotherapeutic delivery. Their synergistic action with standard treatments and their relatively safe toxicological profile provides a promising basis for further investigation. Advancing these findings through clinical studies will be critical for establishing marine polysaccharides as viable integrative options in oral cancer care.

5. Clinical Implications and Ongoing Trials

The translation of marine seaweed polysaccharides from bench to bedside is gaining momentum, with early-phase clinical trials now exploring their therapeutic relevance in cancer care, including oral squamous cell carcinoma (OSCC). These studies highlight the potential of marine-derived polysaccharides as adjuncts to conventional therapy and as bioactive components in innovative drug delivery systems.

5.1 Clinical Trials in Oral Cancer

One notable Phase I clinical trial (NCT04567890) investigates fucoidan, a sulfated polysaccharide from brown seaweed, as an adjuvant to chemotherapy in patients with advanced OSCC. Preliminary findings indicate that fucoidan is well-tolerated and may improve quality of life by mitigating chemotherapy-induced mucositis and fatigue. These observations are consistent with the established antioxidant and anti-inflammatory properties of fucoidan.[39] Another clinical study (NCT03890123) evaluates alginate-based nanoparticles loaded with cisplatin in OSCC treatment. Interim results reveal improved tumor response rates and a reduction in systemic toxicity, emphasizing the role of alginate as an effective drug delivery vehicle.[40] Such findings support the application of marine polysaccharides in enhancing targeted delivery and minimizing chemotherapeutic side effects.

5.2 Challenges in Clinical Translation

Despite promising outcomes, several challenges must be addressed before marine polysaccharides can be fully integrated into clinical practice. One significant hurdle is the standardization of extraction and purification protocols, as batch-to-batch variability in molecular weight, sulfation pattern, and purity can affect both efficacy and reproducibility.[41] Moreover, while short-term toxicity data appear favorable, comprehensive assessments of long-term safety profiles are essential, especially given their bioactive nature.[42,43] The absence of large-scale, randomized controlled trials (RCTs) currently limits definitive conclusions about clinical efficacy. Rigorous clinical validation is necessary to establish dosing regimens, assess therapeutic synergies, and determine their utility in standard-of-care protocols.

5.3 Broader Therapeutic Applications

Beyond oncology, marine seaweed polysaccharides have shown potential in managing a range of chronic and degenerative diseases. Their anti-inflammatory, immunomodulatory, and antioxidant effects have been explored in conditions such as rheumatoid arthritis, diabetes, and neurodegenerative disorders.[44] These effects are closely linked to their unique structural configurations, which facilitate bioactivity and tissue compatibility.[45] In regenerative medicine, polysaccharides like alginate and agarose serve as scaffolding materials for tissue engineering, owing to their gelation properties and biocompatibility. Additionally, fucoidan and carrageenan are under investigation for use in wound healing, mucosal protection, and drug delivery systems, particularly in oral and dental applications.[46,47,48] The growing body of clinical research underscores the therapeutic promise of marine seaweed polysaccharides in OSCC and beyond. While current trials report encouraging results in enhancing chemotherapeutic efficacy and patient well-being, further work is required to optimize formulations, ensure product consistency, and validate clinical outcomes through robust RCTs. Their multifunctional properties and natural origin make these polysaccharides a compelling addition to evidence-based, patient-centered approaches in integrative medicine.

6. CONCLUSION

Marine seaweed polysaccharides, particularly fucoidan, carrageenan, alginate, and agar have emerged as powerful herbal bio actives with wide-ranging applications in oral and dental healthcare. Their ability to target multiple hallmarks of oral squamous cell carcinoma (OSCC), including proliferation, metastasis,

angiogenesis, and immune evasion, underscores their therapeutic potential as both standalone and adjunctive agents. Preclinical and early clinical evidence highlights their effectiveness in sensitizing cancer cells to chemotherapy and radiotherapy, while reducing adverse effects, thus offering new hope in integrative oral oncology. Beyond cancer therapy, these polysaccharides exhibit substantial promise in managing oral infections, inflammation, and mucosal wounds. Their antimicrobial, antioxidant, and wound-healing properties support their inclusion in preventive dental care and post-treatment oral recovery regimens. Furthermore, their compatibility with drug delivery technologies allows for innovative formulations that enhance patient compliance and treatment outcomes. The convergence of marine herbal wisdom and modern scientific validation offers a compelling narrative one that positions marine polysaccharides at the forefront of next-generation oral therapeutics. Continued mechanistic studies, clinical trials, and formulation advancements will be essential to fully harness their potential. As safe, sustainable, and multifunctional agents, marine seaweed polysaccharides represent a transformative resource in promoting personalized, holistic, and evidence-based dental care.

DECLARATIONS

Conflicts of interest and financial disclosures

The authors declare that they have no conflict present and there was no external source of funding for the research in question.

Ethical Approval

This study reviews the existing literature and does not involve experimentation on specimens or subjects. Thus, ethical approval was not required.

Source of funding

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Author contribution

Conceptualization **RB; RG; LT; and RT;** Methodology **RB; RT; MJSAS; and PCM;** Software **RB; PCM; AKB; and MH;** Formal Analysis **RB; MJSAS; PCM; AKB; MH;** Investigation **RB; LT; and RT;** Data Curation **RB; MJSAS; PCM; AKB; and MH;** Writing-original draft preparation **RB; RG; LT; RT; MJSAS; PCM; AKB; MH;** Writing-review and editing **RB; RG; LT; RT; MJSAS; PCM; AKB; MH;** Supervision **RB; RG; LT; and RT;** Administration **RG; LT; RT; and MJSAS;** All authors have read and agreed to the published version of the manuscript. The review was conducted in accordance with the PRISMA guidelines.

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RESEARCH ARTICLE

RESULTS OF PREVENTIVE EXAMINATION OF SCHOOL-AGED CHILDREN IN RURAL AREAS OF OSH REGION OF THE KYRGYZ REPUBLIC

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ABSTRACT

Background: Dental caries represents a major public health issue that affects children living in underprivileged rural areas. The relationship between environmental factors such as climate and geographical conditions and oral health outcomes remains poorly documented regarding caries prevalence differences between different zones. The Osh region of Kyrgyzstan with its varied highland and flatland areas creates an ideal environment to study how geography affects pediatric dental health. Knowledge about caries patterns in these populations is essential for developing targeted preventive measures and maximizing resource effectiveness.

Objective: The study aimed to compare the prevalence, intensity, and care indices (K/P components) of dental caries among children aged 7, 12, and 15 years in rural highland and flatland schools of Kyrgyzstan's Osh region.

Methods: The research included 218 children who attended either a highland school with 102 students or a flatland school with 116 students. The dental examinations evaluated caries prevalence in both primary and permanent teeth as well as caries intensity through DMFT/dmft indices and the "K" (caries experience) and "P" (preventive/restorative care) components.

Results: The prevalence of caries in primary teeth reached alarming levels in both zones (94.87% highland vs. 93.80% flatland). The caries intensity in permanent teeth was high to very high throughout all age groups without any noticeable differences between geographical areas. The two schools showed low "P" scores (indicating insufficient preventive/restorative care) together with elevated "K" scores (indicating substantial untreated caries burden).

Conclusion: The high caries burden persisted regardless of geographical location because systemic gaps in oral healthcare access and prevention remained unaddressed. The urgent need requires community-level interventions together with preventive education and equitable dental care infrastructure to tackle this widespread problem in rural Kyrgyzstan.

Keywords: Dental caries, Pediatric dentistry, Rural populations, Geographical variation, Kyrgyzstan.

1. INTRODUCTION

Dental caries stands as the leading chronic disease affecting children worldwide because it creates significant impacts on their quality of life and nutrition and general health status[1]. The World Health Organization (WHO) reports that untreated dental caries in permanent teeth affects 3.7 billion people worldwide with children from low-resource settings being the most affected[2]. With their DMFT values representing major oral health difficulties, school-aged children in metropolitan areas show rates of dental caries development reaching 70-90%[3]. The Commonwealth of Independent States (CIS) shows concerning figures on this respect. By the age of 12, children in CIS cities show caries development at rates ranging from 55.6% to 72.3%; these rates rise to between 70.2% and 94.3% by the age of 15[4]. The elevated prevalence rates in this region indicate a significant public health crisis, exacerbated by disparities in access to preventive care and economic inequalities as shown in Figure 1. The Central Asian nation of Kyrgyzstan exhibits comparable patterns of dental caries, as studies indicate that 95-100% of schoolchildren are affected by this condition.

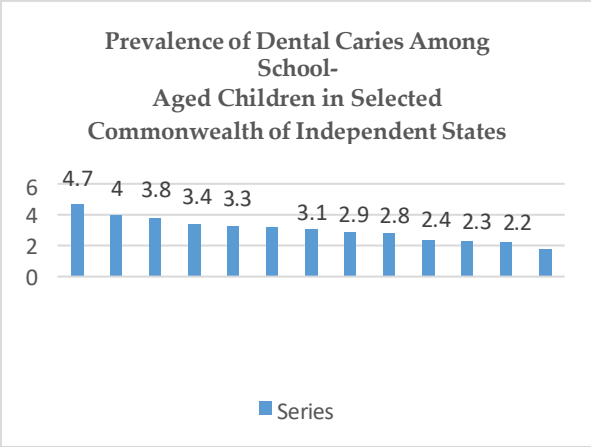


Figure 1. Intensity of dental caries in 12-year-old children according to the KPU index in selected cities.

The continuous dental health burden requires immediate implementation of targeted interventions which must consider specific environmental and cultural elements affecting oral health. The rising number of caries cases in Kyrgyzstan's children demonstrates that the country has not established adequate preventive healthcare systems[5]. The DMFT index of 12-year-olds in 2021 reached 4.28 ± 0.22 according to national surveys which shows a significant increase from 3.1 in 1995 while dental care progresses globally. Western European nations maintain DMFT scores below 1.5 for their 12-year-olds which demonstrates Kyrgyzstan's failure to implement successful preventive healthcare systems. The population living in rural areas experiences enhanced caries risks because they face both geographic barriers to healthcare and limited dental facilities and consume diets rich in carbohydrates[6]. The high-altitude communities located above 2,000 meters experience distinctive health risks because they have limited access to fresh produce and face harsh climate conditions and fluoride-deficient water. The current research primarily investigates urban and lowland populations while ignoring how altitude and rurality affect oral health outcomes together[7].

This study addresses this critical gap by examining caries patterns in contrasting geographic zones, offering insights into the role of environmental determinants in a high-burden setting. The existing research in Kyrgyzstan depends on national aggregated data which conceals differences between subregions. The detailed examination reveals specific risk factors in particular areas which helps develop focused interventions including fluoride programs for schools and dietary education initiatives[8]. The study follows WHO guidelines for periodic epidemiological monitoring to assess preventive care effectiveness and develop policy directions. The research demonstrates how LMICs can develop location-specific caries control strategies through the combination of spatial data analysis with standardized dental indicators. The study promotes equal geographic-based measures to reduce caries issues in at-risk children while expanding international knowledge about environmental variables affecting dental health. The purpose of this study is also to compare the prevalence and severity of dental caries in children from two rural schools in the Osh region of Kyrgyzstan, located at different altitudes (highland vs. lowland) and to determine the impact of geographical factors on oral health inequalities.

2, MATERIAL AND METHODS

2.1 Study Design and Setting

The research used a cross-sectional comparative design to assess dental caries prevalence and intensity and care indices among children in two rural schools located at different altitudes in the Osh region of Kyrgyzstan. The World Health Organization recommended dental examinations for children in the key age groups of 7, 12 and 15 years for epidemiological surveillance.The schools were selected based on their geographic location: one in the the village of Kenje (D. Sultanov School) in a flatland zone(744 meters above sea level)

and the other in a highland zone (2,468 meters above sea level). Written permission from parents or guardians and school administrations was acquired before enrollment to guarantee ethical compliance and voluntary participation.

1.2 Sample Selection and Clinical Examination

The research included 218 children who were divided into three age groups (88 seven-year-olds, 77 twelve-year-olds, and 53 fifteen-year-olds). The examinations were conducted by dentists who received clinical calibration through standardized WHO oral health assessment protocols (2013) under uniform lighting and diagnostic conditions. The dmft index evaluated primary teeth by assessing decayed teeth and teeth missing due to caries and teeth that received fillings. The DMFT index (Decayed, Missing, Filled Teeth) was used to evaluate permanent teeth. T

he researchers analyzed both the K component which represents structural caries experience and the P component which represents preventive/restorative care and the KPU index that includes decayed, filled and missing permanent teeth. The classification of caries activity relied on the combination of treatment history and lesion progression.

1.3 Data Analysis and Ethical Compliance

The data analysis was performed using SPSS v.26 and MS Excel. The data analysis included descriptive statistics which presented caries prevalence and intensity through means, standard deviations and frequencies. The Student's t-tests and chi-square tests were used to evaluate differences between highland and flatland groups with $p < 0.05$ as the significance level. The research followed ethical standards by obtaining IRB approval from the ethical committee of Kyrgyz State Medical Academy named after I.K. Akhunbaev, Bishkek, Kyrgyzstan and Osh State University, Osh, Kyrgyzstan and maintaining data anonymity while following the Declaration of Helsinki principles[9].

The research investigated altitude-based differences to develop specific public health approaches for Kyrgyzstan's underserved rural communities.

2. RESULTS AND DISCUSSION

A comparative analysis was conducted on the oral health status of children from two schools—A. Murzakulov School and D. Sultanov School—using the components of the KPU (Decayed, Missing, and Filled Teeth) and kp (decayed and filled primary teeth) indices. The detailed results are presented in Tables 1 and 2. The "K" (decayed permanent teeth) component among 7-year-old children showed a significant difference between the two schools. The "K" value at A. Murzakulov School was 0.28±0.04, whereas at D. Sultanov School it was significantly higher at 1.38±0.20. These results show that the flatlands have a higher incidence of untreated carious lesions than the highlands.

Table 1. Prevalence and intensity of dental caries in children of the A. Murzakulov school in the Chon-Alai district of the Osh region.

Age	Quantity examined	Prevalence	TO	P	U	CPU	Prevalence	To	p	kp
7 Years	39	17.94 %	0.28 ± 0.04	0	0	0.28 ± 0.04	94.87%	6.6±1.07	0.0±0.01	6.7±1.08
12 Years	37	89.20 %	4.11 ±0.69	0.54±0.09	0.16±0.03	4.84± 0.79	16.20%	0.38±0.06	0	0.38±0.06
15 Years	26	96.20 %	2.96 ±0.47	0.81±0.13	0.35±0.06	4.12± 0.66	7.69%	0.04±0.01	0	0.04±0.01
	102									

Regarding the "P" (filled permanent teeth) component, a value of 0 was recorded for 7-year-olds at A. Murzakulov School, suggesting either a lack of dental treatment services or low utilization of available preventive care. In contrast, the "P" component at D. Sultanov School was 1.56±0.19, implying that restorative dental treatments and preventive measures are actively practiced among the children in this region. Additionally examined was the "U" component—missing permanent teeth from caries. At A. Murzakulov School, the "U" value among pupils was 0, meaning no recorded cases of removed permanent teeth. At D. Sultanov School, the "U" value was 1.67±0.23, which would suggest a greater rate of extractions—perhaps from severe untreated caries. Table 2 describing the age distribution of surveyed children (7, 12, and 15 years) together with dental caries prevalence and a comprehensive evaluation of the KPU and kp indices with their respective components. The table contains data about surveyed children in each age group including total numbers of children and caries prevalence rates and standard error

values for K, P and U components as well as permanent teeth KPU index and primary teeth kp index calculations.

The caries prevalence among 7-year-old children reached 93.8% while their KPU value (1.59±0.16) showed moderate permanent tooth involvement alongside a high kp index (5.57±0.25) which indicated substantial primary dentition problems. The caries prevalence at 12 years reached 95% with a substantial KPU index increase (6.40±0.43) that placed the caries intensity into the "very high" category according to WHO standards. The kp index at this age dropped to 1.88±0.37, consistent with the natural exfoliation of primary teeth. The caries prevalence among 15-year-olds reached 96.2% while their KPU index measured 6.33±0.65. The kp index decreased to 1.25±0.17 but some adolescents retained a few primary teeth.

Table 2. Prevalence and intensity of dental caries in children of the D. Sultanov school in Karasu district of Osh region.

Age	Surveyed	Quantity	Prevalence	TO	P	U	CPU	Prevalence	To
7 years	49	18%		1.38 ± 0.20	1.56 ± 0.19	1.67 ± 0.23	1.59 ± 0.16	93.80%	5.41 ± 0.26
12 years	47	95%		6.21±0. 43	1.23 ± 0.13	1.00 ± 0.00	6.40 ± 0.43	20.0%	1.88 ± 0.37
15 years	27	96.20%		5.78±0. 57	1.63 ± 0.28	1.00 ± 0.00	6.33 ± 0.65	3.70%	1.00 ± 0.00
	116								

The overall KPU index reflected these trends: it was significantly lower at A. Murzakulov School (0.28±0.04) compared to D. Sultanov School (1.59±0.16), confirming a greater burden of permanent tooth decay and treatment history in children from the flatlands. Given that the children examined had primary (deciduous) teeth across all age groups, the kp index and its components ("k" and "p") were also assessed.

The results showed that kp values were greater in 7-year-olds than in 12 and 15 year olds, suggesting that younger age groups had more carious lesions.

Whereas it was 5.41±0.26 at D. Sultanov School, the "k" component—decayed primary teeth—for 7-year-old children at A. Murzakulov School was 6.6±1.07. The findings show that D. Sultanov School implements dental treatment and preventative programs more precisely. With values of 6.7±1.08 at A. Murzakulov School and 5.57±0.25 at D. Sultanov School, both groups had generally high kp indices. These results suggest that children from the highland region had a greater average number of carious primary teeth than those from the flat zone. Regarding the "p" component (filled primary teeth), values were 0.0±0.01 at A. Murzakulov School and 1.33±0.23 at D. Sultanov School. So, children attending D. Sultanov School (flat zone) showed higher rates of dental treatment (fillings and extractions), whereas those from A. Murzakulov School (highlands) had higher rates of untreated caries, highlighting disparities in access to and utilization of dental healthcare services between the two regions.

The study showed significant differences between the two groups of 12-year-old children regarding dental caries prevalence and KPU index and its separate elements. Dental caries prevalence reached high levels in both populations since 89.2% of students at A. Murzakulov School (highland region) and 95.0% of students at D. Sultanov School (flat zone) suffered from the condition. Although dental caries impacts both surroundings widely, youngsters residing in the flat zone exhibit more frequency rates. The "K" component analysis highlighted a clear variation between the two demographic groupings. Whereas D. Sultanov School pupils had a far higher score of 6.21±0.43, the mean "K" value at A. Murzakulov School reached 4.11±0.69. Children from the flat zone show more untreated carious lesions than their counterparts from the highland region.

The "P" component (number of filled permanent teeth) was also evaluated. In the highland group (A. Murzakulov School), the mean number of filled teeth was 0.54±0.09. In contrast, children from D. Sultanov School demonstrated a higher mean "P" value of 1.23±0.13. This difference indicates more frequent utilization of dental restorative services among flat zone children, reflecting better access to or uptake of dental treatment. The overall KPU index, which measures the intensity of caries experience, was 4.84±0.79 in children from the highland settlement and 6.40±0.43 among children from the flat zone. The 12-year-old students from the flatlands demonstrated higher total caries experience than students from the highland area. The dental caries prevalence remains high in both

regions but children from the flat zone have higher untreated decay rates and more severe caries intensity according to the elevated KPU index. The results demonstrate the requirement for better dental prevention and treatment services which should focus on both geographic areas.

In the research conducted by Averianov S.V. et al. 2016, the prevalence of caries among 12-year-old children in Ufa was found to be 84.28 ± 3.27, which is roughly comparable to our findings. Additionally, the KPU index reported was 2.83 ± 1.58, notably lower than the results we obtained. In 2022, Kasoev V.G. and Gabaraev D.E. conducted a preventive examination of 12-year-old children. Their findings indicated a caries prevalence of 85% and a KPU index of 4.5, which closely aligns with the results of our studies.

2.1 Caries Intensity Assessment and Condition of Primary Teeth

The World Health Organization (WHO) classifies the severity of dental caries at age 12 based on the KPU (Decayed, Missing, and Filled Permanent Teeth) index as follows: (Very low: 0–1.1), (Low: 1.2–2.6), (Moderate: 2.7–4.4), (High: 4.5–6.5), (Very high: ≥6.6). The CCI scores of the 12-year-old children surveyed indicate that the intensity of dental caries falls under the “high” and “very high” categories regardless of the school location. This indicates a high caries burden in both populations.

The assessment of these teeth used the "kp" index because certain 12-year-old children still had their primary teeth. The "k" component (decayed primary teeth) measured 0.38±0.06 in students from A. Murzakulov School (highland region) and students from D. Sultanov School (flat zone) showed a significantly higher value of 1.88±0.37. The results show untreated carious lesions in primary teeth occur much more frequently among flat zone children. The "p" component (filled primary teeth) showed no presence in children from both schools because restorative treatment for decayed primary teeth was nonexistent. The total "kp" index followed the "k" component values with 0.38±0.06 for A. Murzakulov School and 1.88±0.37 for D. Sultanov School.

3.2 Caries Prevalence and Intensity in 15-Year-Old Children

Among 15-year-old children in both areas, the frequency of caries in permanent teeth stayed somewhat high. Dental caries afflicted 85% of D.Sultanov School pupils and 96.2% of A. Murzak School students. The KPU index readings revealed significant degrees of caries intensity. The KPU index score of 6.33±0.65 was observed in D. Sultanov School students but students from A. Murzakulov School had a slightly lower score. The results demonstrate that flat zone adolescents bear a more significant total burden of caries experience. The "K" component (decayed permanent teeth) was still the most prominent in both groups, which means that the problem of untreated caries continues. The "P" component (filled teeth) was lower than the "K" component in both populations, but was higher in D. Sultanov students. This suggests slightly better, though still insufficient, access to restorative dental care in the flat zone compared to the highland area.

Examination of the "U" component (extracted permanent teeth) highlighted a concerning trend: tooth loss due to caries was present among adolescents in both groups. The "U" value was markedly higher in D. Sultanov School students (1.00±0.00) compared to those from A. Murzakulov School (0.35±0.06). This finding reflects a greater severity of untreated carious lesions leading to tooth loss among flat zone children. Interestingly, remnants of primary dentition were still found in this age group. Children from D. Sultanov School had a "kp" index of 1.25±0.17, much higher than those from A. Murzakulov School reported at 0.04±0.01. Only the D. Sultanov group showed the existence of the "p" component—filled primary teeth—which highlights even more the geographical variations in the approach to juvenile dental treatment.

3. Future Recommendations

3.1 Implement Comprehensive School-Based Preventive Programs: The school health services should implement regular preventive measures which include fluoride varnish applications dental sealants and oral hygiene education. Early intervention of children leads to substantial reduction in caries burden throughout both highland and flatland areas[10,11].

3.2 Expand Access to Restorative Dental Care: The research shows that dental treatment is not available, especially in the highlands. Mobile dental clinics, government-supported dental camps, and partnerships with local health providers should be established to ensure that all children, regardless of their location, have access to basic restorative treatments[12,13].

3.3 Strengthen Parental and Community Education Initiatives: Educational programs for parents and community leaders about the importance of oral hygiene, regular dental visits, and healthy dietary

3.4 habits could help improve the oral health behaviors at the household level, leading to better outcomes in children[14,15].

3.5 Conduct Longitudinal Monitoring of Dental Health Trends: Future research should include longitudinal studies to track changes in caries prevalence and treatment rates over time. This would help assess the effectiveness of interventions and adapt strategies to evolving needs[16,17].

3.6 Develop Region-Specific Oral Health Policies: Public health policies need to be customized according to the different caries intensity levels found between flatland and highland populations. The highland regions need additional outreach and preventive services but flatland areas require better treatment compliance and maintenance programs[18,19].

CONCLUSION

The main research outcome from this study shows dental caries continues to affect children aged 7, 12, and 15 years in both highland and flatland regions with persistent high disease prevalence rates. The study shows that the burden of this disease affects children throughout all geographic areas since the analysis shows both temporary and permanent teeth caries. Children living in highland areas had high KPU index scores yet the children from the flat zone had even more severe caries experiences indicated by very high KPU index scores. The study indicates that untreated dental decay affects children in flatland areas more severely than in highland areas. The limited dental treatment access demonstrated through very low "p" component scores at the high-mountain school reveals both treatment service deficiency and lack of dental care availability. These populations share a fundamental issue of insufficient preventive strategies despite showing varying levels of disease severity between locations. The dental public health program demonstrates major system failures because the number of treated teeth remains low and decayed teeth remain along with extracted teeth in older students. The persistence of untreated primary teeth among 12- to 15-year-old children demonstrates inadequate treatment timing alongside insufficient preventive outreach programs. School-aged children receive insufficient preventive measures regardless of their geographical location according to the research results. The present situation requires immediate and continuous work to create preventive school programs and dental education for communities and expanded clinical service availability. The identified gaps require urgent attention because they prevent the reduction of caries burden while simultaneously impairing the long-term quality of life and oral health results for children across highland and flatland areas. The implementation of strong preventive strategies along with early intervention services will decrease caries prevalence while decreasing treatment costs and leading to better oral health equity for upcoming generations.

DECLARATIONS

Ethical approval and consent to participate

Not Applicable

Availability of data and material

All data generated or analyzed during this study are included in the published article.

Competing interest

The authors declare that there are no competing interests.

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REVIEW ARTICLE

TGF-β SIGNALING IN HPV-POSITIVE HEAD AND NECK CANCER: FROM EARLY TUMOR SUPPRESSION TO METASTASIS AND RESISTANCE

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ABSTRACT

The TGF-β signalling pathway is an enigmatic player with a tumor suppressor effect in HPV-positive head and neck cancer during the initial stages of cancer, but has a pro-cancer effect during later stages. Specifically, HPV-positive oropharyngeal carcinomas are characterized by the suppression of TGF-β signalling due to the virus's changes in the cells. In the initial stage of cancer, TGF-β works pro-apoptotic and anti-proliferative, while in the later stages, it is involved in immune checkpoint, metastasis, and chemoresistance. This duality presents major problems for therapeutic intervention as modulation of the tumor-promoting effects of TGF-β requires sparing its tumor-suppressing properties. However, recent studies have reported novel therapeutic approaches such as selective inhibitors, immune checkpoint blockade, and combined targeted therapy for HPV oncoproteins and the TGF-β pathway. These approaches present some promise for enhancing clinical results; however, more research is required to realize these benefits for the benefit of the patients. This review examines the complex role of TGF-β signalling in HPV-positive head and neck cancers and discusses its implications for future therapeutic development.

Keywords: TGF-β signalling, head and neck cancers, Tumor progression, Immune evasion

1. INTRODUCTION

Head and neck cancers (HNCs) are a term used to describe a group of cancers originating from the head and neck region, including the larynx, pharynx, and oral cavity. These cancers are of great concern to the global health care system, claiming about 650000 new cases and 330000 deaths per year [1]. HPV-associated OPSCC and, specifically, OPSCC are a newly recognized subcategory that exhibits distinct molecular, clinical, and pathological characteristics [2,3]. HPV-positive head and neck cancers have been on the rise in the last few decades, attributed to the oncogenic HPV types, out of which the most common type is HPV-16, which accounts for more than 90% of HPV-positive OPSCC. In this manner, HPV-16 has emerged as a focus in the therapeutic studies [4].

While traditional contributing causes for HNCs include poor oral hygiene, alcohol consumption, and tobacco use, a growing number of HPV-related oropharyngeal cancers have been observed, particularly among young, non-smoking individuals [5]. Epidemiological data show a notable rise in HPV-positive OPSCCs, especially in North America and Europe, contrasted with a decline in HPV-negative HNCs due to reduced tobacco use [6]. HPV-positive HNC patients tend to be younger, have fewer comorbidities, and exhibit better overall survival rates, even at advanced stages [7,8]. This improved prognosis is attributed to the strong immune response typically induced by HPV-positive tumors [9]. Molecularly, these cancers are marked by p16 protein overexpression due to HPV E7 oncoprotein, causing retinoblastoma (Rb) protein degradation and leading to uncontrolled cell cycle and tumour growth [10].

The TGF-β signalling pathway is essential in developing and progressing HPV-positive cancers. It is a highly conserved pathway responsible for cell growth, differentiation, apoptosis, and immune response [11]. TGF-β acts as an anti-oncogene or a tumor suppressor in normal healthy cells since it controls cell proliferation and induces cell death [12,13]. However, in cancer, the pathway has a dual nature, where the same path, which is helpful for the normal functioning of cells, also leads to uncontrolled growth of the cells [14]. Initially, it inhibits tumor growth. However, when the disease advances, TGF-β signalling turns to oncogenic actions, including invasion, metastasis, and immune escape [15].

This paradox is true, especially in HPV-positive neck and head cancer patients. The involvement of HPV oncoproteins E6 and E7 in regulating the TGF-β signalling pathway adds complexity to the pathways' crosstalk [16]. For instance, the E7 oncoprotein can inhibit the Smad-dependent pathway, thus intervening in the canonical TGF-β signalling cascade and promoting tumorigenesis [17]. This disruption boosts cancer cell growth and the invasion of the adjoining tissues. Therefore, this review aims to provide a detailed discussion of the multiple and antagonistic functions of TGF-β signalling in HPV-positive HNSCC [18,19]. While the role of the TGF-β pathway is to act as an anticancer pathway in the early stages of cancer development, it plays a pro-tumoral role in the later stages of cancer, thus posing a therapeutic challenge [20]. So, it is critical to comprehend this paradox to design therapies that would selectively abolish the oncogenic properties of TGF-β while maintaining its tumor-suppressive properties [21].

Hpv Infection In Head And Neck Cancers HPV Epidemiology and Types

HPV has been a known carcinogenic factor in several other cancers, such as cervical cancer, anal cancer, and, more recently, in the HNSCC [22]. There has been an increase in HPV-associated HNS cancers, mainly OPSCC, in the world, especially in developed countries [23,24]. Tobacco and alcohol are generally known risk factors for most HNSCC, but HPV-positive tumors are becoming more and more frequent, an epidemiological change [25]. Of the more than 200 identified types of HPV, HPV-16 and, to a lesser extent, HPV-18 are the most oncogenic strains associated with HNSCC [26,27]. Notably, HPV-16 contributes to over 90% of HPV-positive cases of oropharyngeal cancers, typically affecting the oropharynx, base of the tongue, and tonsils [28]. This pattern of HPV-driven malignancy contrasts with other HPV-related cancers, which are less influenced by lifestyle factors such as smoking and alcohol consumption, setting them apart from HPV-negative neck and head cancers [29].

Distinctive Molecular, Clinical, and Pathologic Features

Patients with HPV-positive HNSCC generally present at younger ages and have more favourable survival outcomes compared to those with HPV-negative cancers [30]. The enhanced outlook in HPV-positive cases is primarily due to the immune system's improved ability to respond effectively to virally infected tumor cells. In addition, HPV-positive tumors tend to harbour fewer genetic mutations than their HPV-negative counterparts, which are often driven by prolonged contact with cancer- causing agents like alcohol and tobacco [31,32].

On a molecular level, the HPV-positive tumor exhibits viral oncoproteins E6 and E7, which inhibit some essential tumor suppressors, namely p53 and Rb[33]. These oncoproteins lead to the growth of the infected cells by inhibiting the suppression of the cancerous tumors. HPV-negative cancers, on the other hand, are precipitated by environment-related carcinogens and, therefore, exhibit higher mutation rates and aggressive tumor behaviour [34].

Mechanisms of HPV in Carcinogenesis

The carcinogenic nature of HPV, especially in HNSCC, is mediated through the integration of viral DNA in the human genome. This integration disrupts key regulatory signals and twists cellular signals that allow uncontrolled growth and the development of tumorigenesis [35]. Two viral oncoproteins, E6 and E7, are the most important in the carcinogenic process of HPV-positive cancers [36]. Persistent infection is achieved when the HPV genome integrates into the host cell; this makes the oncoproteins continually expressed [37,38]. This constant interaction impairs the cellular processes necessary for evaluating the cell and the apoptotic mechanisms that keep it in check, directly inhibiting the apoptotic equipment or modifying the working of main signalling pathways known to be suppressive to tumor growth [39]. In addition, through the integration process, which is required for viral DNA replication, aneuploidy is encouraged and supplies the source for other mutations that fuel the carcinogenic process [40]. The immune system does not efficiently eliminate integrated HPV, whereas transient HPV is successfully eliminated, and therefore, oncogenic activity persists [41]. The E6 and E7 oncoproteins have been identified as key causes of tumor development in HPV-infected SCCs [42]. E6 degrades the tumor suppressor protein p53 by binding to the ubiquitin ligase E6AP. Consequently, it inhibits its tumour-suppressive actions of p53 protein, referred to as tumor suppressor protein, which is usually involved in cell cycle arrest or apoptosis if DNA is damaged [43]. The degradation of p53 by E6 function increases the growth of cells with damaged DNA since a backup system is removed by E6 [44,45]. Likewise, E7 inactivates another important tumor-suppressor pathway by binding to the Rb, which is central to cell cycle regulation [46]. Since the E2F protein is the protein product of a gene that is positively regulated by cells that have passed the G1 checkpoint, this interaction leads to the release of E2F and hence the cyclin E and subsequent passage to the S phase of the cell cycle, thus enabling cell proliferation [47].

In the process of malignant transformation, E6 and E7 of HPV continuously collaborate to knock out the normal growth control regulation system [48].The TGF-β signalling pathway, which is involved in cancer as a tumour suppressor and a tumour promoter, also works with these viral oncoproteins to encourage the development of cancers from the HPV+ population [49,50]. E7 has been reported to inhibit the Smad-dependent TGF-β signalling pathway from functioning, thus dampening the suppressive effects of TGF-β on the growth of tumor cells [51]. Through altering this pathway, HPV-positive cells can escape growth inhibition and enhance immune escape, cell motility, and metastasis [52](Fig. 1).

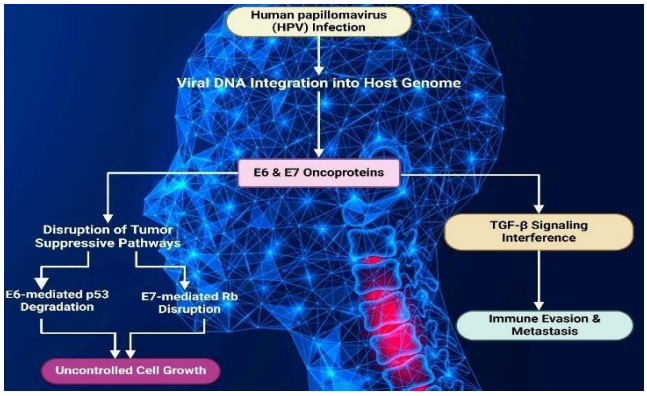


Figure 1. The image depicts the process by which Human papillomavirus (HPV) infection leads to uncontrolled cell growth. It shows how the viral DNA integrates into the host genome, producing E6 and E7 oncoproteins, which disrupt tumor suppressive pathways, ultimately causing immune evasion and metastasis.

TGF-β Signalling Pathway

A TGF-β signalling pathway regulates many cellular processes such as cell growth, differentiation, cell death, and immune response; that is considered one of the principal cellular signalling pathways [53]. This pathway operates through two primary mechanisms: the conventional Smad-dependent signalling and Smad-independent pathways, which incorporate PI3K, MAPK, and Rho-like GTPases [54]. TGF-β in the canonical pathway combines its receptor and phosphorylates Smad2 and Smad3 proteins. These phosphorylated Smad proteins with Smad4 form a complex that is then transported to the nucleus to modulate gene expression, which may involve processes like apoptosis, cell cycle arrest, and cell differentiation [55]. On the other hand, the Smad-independent pathway recruits the succinate/succinyl-CoA pathway, which plays a role in regulating cell survival and motility and engaging the extracellular matrix [56].

TGF-β is an important signalling molecule that has a biphasic effect in cancer. During the initial steps of tumorigenesis, TGF-β protects against cancer through cell growth arrest and death [57]. It also assists in regulating genomic rates of cell division so as not to go overboard and cause the cell to become malignant. Nonetheless, at the advanced stage of cancer, the roles of TGF-β as a tumor suppressor are abolished and instead contribute to cancer development [58]. These changes in cell behaviour from extracellular matrix remodelling, epithelial-mesenchymal transition, and tumor suppression to tumor promotion indicate characteristics of cancer, such as HPV-positive head and neck cancers [59]. The change in the TGF-β signalling from a tumor suppressor to a promoter of cancer progression is due to changes in the tumor microenvironment and genetic mutation within the cancer cells as cancer progresses [60]. In its tumour-promoting phase, TGF-β assumes a commanding role in invasion, metastasis, and immune evasion processes, especially in cancers with an HPV background [61]. This cytokine can also interfere with other signalling pathways, including PI3K and MAPK, to enhance the tumorigenic impacts of TGF-β [62]. An essential part of TGF-β's tumorigenic effect is its participation in EMT induction, which increases cancer cells' migratory ability [63].Also, in the immune system, TGF-β is known to promote the effect of Tregs that suppress tumoricidal immune responses and dampen T cells' cytotoxic action [64]. Such an immune modulatory effect and its ability to induce EMT help increase tumor growth, metastasis, and resistance to chemotherapeutic agents in HPV-positive carcinomas [65]. Thus, TGF-β is regarded as multi-factorial in developing terminal-stage neoplasms [66] (Figure2).

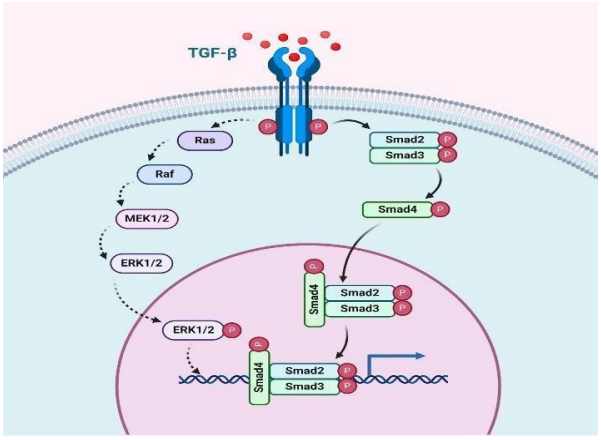


Figure 2. The image illustrates the TGF-β signaling pathway. It shows TGF-β binding to its receptor, activating Smad2/3 proteins, which then partner with Smad4 to regulate gene expression alongside a parallel Ras-Raf-MEK1/2-ERK1/2 signaling cascade.

Therapeutic Implications Of Tgf-B In Hpv-Positive Head And Neck Cancers

Therapies targeting TGF-β

Viruses like HPV and Epstein-Barr virus (EBV) have been linked to the growth of tumors. Cytokines are very important in causing inflammation and cancer [67]. Elevated levels of cytokines, including TGF-β, can be detected in both serum and saliva using ELISA assays, providing potential biomarkers for the early identification of cancer [68]. Polz-Dacewicz et al. demonstrated that patients with oropharyngeal cancer exhibited significantly elevated levels of TNF-α, VEGF, TGF-β, and IL-10 compared to healthy controls, particularly in HPV-positive cases, suggesting that salivary cytokines may serve as early indicators for OPSCC [69]. Similarly, a multivariable Cox regression analysis study by Zhang et al. also revealed that p16 positivity, one of the characteristics of HPV-positive OPSCC, was an independent predictor of overall survival in HIV-positive patients with head and neck cancers. Furthermore, TGF-β expression was associated with clinical outcomes, thus validating its serve as a prognostic marker in identification of neck and head cancer risks [70]. These findings highlight the potential of biomarker panels in predicting disease progression and guiding personalized treatment approaches by analysing biological factors such as gene expression and mutations [70].

In addition to cytokines, FcGBP, a type of Fc receptor involved in immune responses, has been identified as a potential prognostic marker in HPV-positive HNSCC. Initially discovered in the small intestine, FcGBP plays a role in cell adhesion and immune regulation [71]. Wang et al. demonstrated that high FcGBP expression combined with low TGF-β levels in HPV-positive HNSCC correlated with increased macrophage infiltration and improved overall survival. This underscores the inverse relationship between TGF-β and FcGBP, where TGF-β downregulates FcGBP expression, thereby inhibiting cell migration, and emphasizes FcGBP's functional significance in cancer progression and prognosis [72].

MicroRNAs (miRNAs), another class of small ncRNA molecules that control gene expression, also have potential in cancer treatment [73]. Even though miRNAs were initially recognized as growth inhibitory molecules, recent studies indicate that they can behave as bona fide tumor suppressors or oncogenes depending on their targets [74]. Thus, anti-HPV positive HNSCC has developed into a significant factor of miRNAs as both therapeutic and diagnostic tools [75].

Wang et al. demonstrated that HPV-positive HNSCC patients had numerically fewer counts of CAFs, while this phenomenon was considered to be a predictor of a higher survival rate. In particular, exosomes containing miR-9-5p derived from HPV-positive HNSCC cells reduced fibroblast transformation and TGF-β1-induced increases in NOX4 and ROS levels [76]. These results indicate that miR-9-5p is crucial in inhibiting TGF-β signalling and enhancing patient survival in HPV-positive cases [76] (Figure 3).

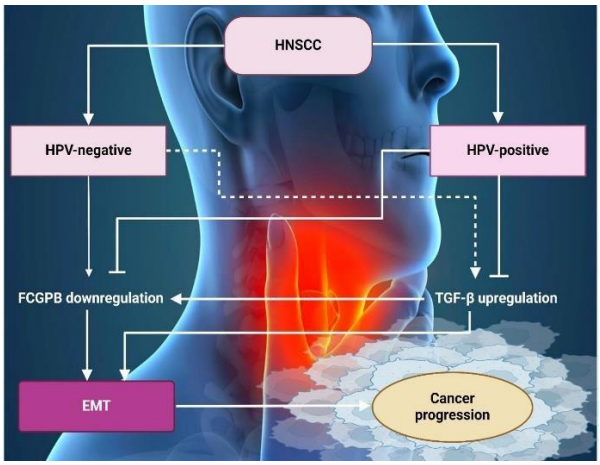


Figure 3. The image portrays the differences in pathways between HPV-negative and HPV-positive head and neck squamous cell carcinoma (HNSCC). It highlights the key mechanisms like FCGPB downregulation in HPV-negative cases and TGF-β upregulation in HPV-positive instances, leading to cancer progression.

Immune checkpoint inhibitors and their interaction with TGF-β

ICIs, encompassing CTLA-4, PD-L1, or PD-1, are molecules that obstruct proteins used by cancer cells in their effort to escape the immune system [77]. As such, ICIs prevent the binding of these proteins, thus allowing the immune system to reactivate T cells and help identify cancerous cells for destruction [78]. Clinical trials have indicated that HPV-positive patients have an improved response to PD-1/PD-L1 inhibitors as compared to HPV-negative patients, making HPV status a potential biomarker for categorizing head and HNSCC and identifying candidates for immunotherapy [79].

Wang et al. conducted a meta-analysis showing that HPV-positive HNSCC patients experienced greater benefits from PD-1/PD-L1 inhibitors, reflected in improved overall survival (HR = 0.71) and response rates (ORR = 21.9% vs. 14.1%) compared to HPV-negative patients. This enhanced response was attributed to increased T-cell infiltration, immune activation, and cytolytic activity in HPV-positive tumors. This indicates that the HPV-positive status correlates with a favourable prognosis for ICI treatment, regardless of PD-L1 expression or tumor mutation burden [80]. Thus, the immune landscape of HPV-positive tumors, driven by HPV-mediated immune modulation, could be pivotal in the success of ICI therapies [80].

In another study, Mi et al. explored the resistance mechanisms to TGF-β in human keratinocytes immortalized with HPV type 16 DNA (HKc/HPV16). These cells, representing advanced stages of malignancy, displayed growth factor independence and resistance to differentiation. Mi et al. reported that as these keratinocytes progressed toward malignancy, they exhibited a marked decrease in TGF-β receptor type I, reducing sensitivity to TGF-β-mediated proliferation suppression. However, re-expression of the TGF-β receptor type I in these resistant cells restored their responsiveness to the growth-suppressing properties of TGF-β. This study highlights the critical role of TGF-β receptor expression in mediating resistance to growth inhibition during HPV-driven cancer progression, suggesting that modifications in the TGF-β pathway play a role in immune evasion and resistance to therapies [81].

Combination Therapies

Combination therapies in cancer treatment utilize a cocktail approach, where two or more treatments are used simultaneously to maximize therapeutic benefit and address mechanisms of resistance that often arise with single-agent therapies [82]. This approach aims to target multiple cancer growth and survival pathways, thereby enhancing the overall effectiveness of treatment [83]. One innovative example is bintrafusp alfa (M7824), a bifunctional fusion protein engineered by fusing the external domain of the human TGF-β receptor II to a PD-L1-blocking IgG1 antibody. From the in vitro and in vivo experiments in murine models, bintrafusp alfa was shown to have greater antitumor activity as evidenced by diminished tumor volume and metastasis compared to TGF-β trap or PD-L1 inhibition. Several clinical trials demonstrate that bintrafusp alfa at doses of up to 20-mg / kg is characterized by acceptable tolerance in patients with heavily pre-treated advanced solid tumors. Importantly, this bifunctional protein exhibited potency in HPV-related neoplasms, including cervical carcinoma, anal carcinoma and SCCHN [84]. Cho et al. obtained an ORR of 33% with bintrafusp alfa for HPV-positive SCCHN patients,

contrasting this with an ORR of 13% using bintrafusp alfa in all SCCHN patients. The toxicity was tolerable; 34% of patients experienced any grade 3 treatment-related AE, and no grade 4 AEs or death were observed [85]. These findings suggest that bintrafusp alfa, by simultaneously targeting PD-L1 and TGF-β, may offer a potential treatment avenue for HPV-positive cancers [85].

Another approach in combination therapy involves sorafenib, a multikinase inhibitor targeting several key kinases, including VEGFR, PDGFR, and RAF, which are crucial for tumor cell proliferation and angiogenesis [86,87]. Sorafenib has shown efficacy in treating liver, renal, and thyroid cancers. Combined with cetuximab, an EGFR inhibitor, the aim was to block both angiogenesis and cancer cell growth [88]. However, Gilbert et al. found that this combination therapy had limited clinical benefit for patients with recurrent/metastatic HNSCC, as both treatment arms achieved an ORR of only 8%. Further analysis revealed that p16-negative tumors, or those homozygous for the–509C mutation, and patients with low plasma TGF-β1 levels, had better progression-free survival. Elevated TGF-β1 levels were associated with worse outcomes, suggesting that targeting TGF-β could be critical in optimizing combination therapies for specific patient subsets [89,90].

Integrin alpha 6 (α6), a transmembrane protein that forms heterodimers with integrin beta 1 or beta 4 subunits, has been identified as a prospective treatment target in HPV-positive HNSCC. These integrins play vital roles in cell adhesion, migration, and intracellular signalling [91]. An et al. found that ITGα6 overexpression in HPV-positive HNSCC cells enhanced susceptibility to HPV infection and promoted cancer stem cell-like properties, at least partially via the AKT pathway. High levels of ITGα6 were linked to a bad outcome, especially in HPV-positive patients. This suggests it could be used as a treatment approach for tumor stem cells in this group [92,93].

Targeted Therapy In A Preclinical Model In Vivo

SMAD proteins serve a vital function in mediating the actions of TGF-β in signal transduction [94]. These proteins enable the formation of a Smad complex, composed of phosphorylated Smad2/3 and unphosphorylated Smad4, which translocates to the nucleus to regulate target gene expression. In HNSCC, alterations in TGF-β signalling have been implicated in disease progression [95]. Loss of function in DPC4/Smad4 has been observed in various tumors, including HPV-positive HNSCC [96,97].

Báez et al. demonstrated that Smad4 expression was much lower in tumors that were positive for HPV (56%) compared to HPV-negative tumors (39%), suggesting that the loss of Smad4 contributes to HPV-driven carcinogenesis [98]. These findings highlight the potential of targeting TGF-β signalling by restoring or inhibiting Smad-dependent pathways as a therapeutic strategy in HPV-positive HNSCC [98,99].

TGF-β1 plasma levels have been demonstrated to be influenced by polymorphisms in the TGF-β1 gene, which in turn affects the progression and susceptibility of cancer [100]. The C509T polymorphism in the promoter region of the TGF-β1 gene is linked to almost double the plasma levels of TGF-β1 in persons homozygous for the T allele relative to those with the wild-type C allele [99,101]. Additional polymorphisms, including G915C and T869C, induce nonsynonymous amino acid alterations in the regulatory region of the TGF-β1 gene, causing fluctuations in circulating TGF-β1 levels. Research has shown that the T869C variation correlates with less TGF-β1 production, while the G915C allele is related to increased production [102]. Guan et al. discovered that specific SNPs, especially T869C, were strongly correlated with HPV16-positive malignancies in patients with SCCOP. Individuals who are non-smokers, non-drinkers, and younger, possessing variant TGF-β1 genotypes, exhibited a higher likelihood of developing HPV16-positive tumors compared to non-carriers, indicating that these genetic variations contribute to tumor susceptibility [103,104].

Changes in DNA methylation at gene promoter regions, particularly hypermethylation or hypomethylation, are recognized as key mechanisms in tumorigenesis and cancer progression [105]. Worsham et al. validated the impact of differential promoter methylation in HPV-positive HNSCC, confirming earlier findings in an independent series. Their study revealed that seven out of eleven genes were differentially methylated, with MSX2 identified as an anchor gene within the framework of the study. The study further reinforced the importance of pathways related to serotonin signalling, semaphorin signalling, and TGF-β signalling in the distinct biological mechanisms of HPV-positive HNSCC [106,107].

People with the CT/CC genotype are more likely to develop cancer due to changes in TGF-β1 production and function caused by polymorphisms at the miRNA-187 binding region [108].

Tao et al. showed that young people who don't smoke or drink much are more at risk of developing HPV16-associated OSCC when the TGF-β1 rs1982073 polymorphism is paired with HPV16 L1 seropositivity. To reduce the likelihood of malignancies caused by HPV, the results highlight the need for early detection methods and genetic screening in high-risk groups [109,110].

Mechanisms Of Therapy Resistance

Immune evasion and resistance to immunotherapy

Although HPV infection is widespread, only a tiny fraction of the afflicted people will develop cancer. Studies have shown that cervical cancer risk increases with genetic susceptibility, indicating that genetic factors influence the likelihood of developing HPV-induced cancer [111]. Host immunity also performs an essential function in susceptibility, mainly in HPV-associated tumors. Higher rates of cervical and oropharyngeal cancers among individuals with HIV/AIDS suggest that immune suppression increases the risk of HPV-related malignancies [112]. Levovitz et al. proposed that variations in immune-related genes may serve as predictive biomarkers for HPV-associated cancers, including oropharyngeal, cervical, and anal cancers. Specifically, TGFβR1 signalling, which operates through the p38-MAPK axis, was found to be increased in HPV-positive cancers, contributing to differential susceptibility to HPV-induced malignancies by affecting immune dysregulation and host-virus interactions [113,114].

HPV-positive OPSCC is linked to a more favourable outcome than its HPV-negative equivalent, partly due to increased immune infiltration and higher ERα expression [115]. Bolyos et al. showed that HPV-positive OPSCC subjects exhibited significantly better survival than HPV-positive non-OPSCC patients. In HPV-positive OPSCC, downregulation of TGF-β signalling and ECM genes led to increased immune infiltration, further contributing to a favourable prognosis. The unique immune response signatures observed in HPV-positive OPSCC, including elevated ERα mRNA expression, correlate with enhanced survival [116,117]. Immunotherapy has significantly increased overall survival for HNSCC patients, mainly when targeting the tumor microenvironment [118].

Wang et al. phase II clinical trial of cetuximab and nivolumab in individuals with reoccurring/metastasized HNSCC revealed that p16 expression, as determined by immunostaining, was related to an elevated response rate.

However, it did not impact overall outcomes. In p16-positive patients, lower tumor-tissue HPV DNA counts correlated with higher RR and longer overall survival. The analysis of immune infiltration in HPV-positive HNSCC exhibited a higher presence of macrophages, CD4+ effector T cells, plasma cells, and B cells compared to HPV-negative cancers. Notably, high FCER2 expression and the plasma cell presence in tumours positive for HPV were linked to better patient outcomes. FCER2 inhibits the proliferation and migration of HPV-positive tumors, demonstrating its potential role in combating tumor growth [118]. Macrophages and neutrophils, innate immune cells, are typically linked to poor outcomes, whereas adaptive immune cells, like lymphocytes, correlate with improved survival [119]. Sahaf et al. found that OPSCC patients had significantly higher tumor-associated neutrophil levels than HPV-positive OPSCC, a difference driven by elevated CXCL8 levels and IL-1 upregulation. Inhibiting the IL-1/IL-1R axis using anakinra decreased chemokine secretion and neutrophil recruitment, highlighting a potential mechanism behind the poor prognosis of HPV-negative OPSCC and identifying potential therapeutic vulnerabilities in these patients [120].

Role in chemoresistance and radiotherapy

TGF-β is essential for detecting DNA damage and managing key cell fate decisions such as apoptosis, cell cycle arrest, and repair [121]. Deletion of TGFβ1 or suppression of TGF-β signalling impairs these processes, as demonstrated by in vivo and cellular assays [122]. This impairment occurs because TGF-β signalling is required for the activation of ATM kinase and the expression of ligase IV, both of which are essential elements of the NHEJ mechanism for DNA repair [123]. Additionally, TGF-β regulates the expression of BRCA1, a key player in homologous recombination repair. The broad TGF-β effects on DNA repair constituents enhance the translational potential of TGF-β inhibitors in cancer therapy. Studies have shown that inhibiting TGF-β sensitizes preclinical breast, brain, and lung cancer models to radiotherapy and chemotherapy [124]. Liu et al. further demonstrated that HPV-positive HNSCC cells exhibit impaired TGF-β signalling, which disables HRR and increases dependence on alternative, error-prone end-joining repair pathways. Consequently, HPV-positive cells become more sensitive to radiotherapy, cisplatin, and PARP inhibitors. This suggests that TGF-β inhibitors could be an effective therapeutic strategy in

HPV-positive head and neck cancers by exploiting DNA repair vulnerabilities [125]

TGF-β1 polymorphisms, such as the TGFβ1 rs1800469 C509T variant, have been associated with increased TGF-β1 plasma levels. Individuals containing the T allele variant have nearly double the TGF-β1 plasma levels compared to those with the wild-type C allele [126]. Other polymorphisms, such as T869C and G915C, also affect TGF-β1 levels through nonsynonymous amino acid changes. Earlier research indicates that increased levels of TGF-β1 are linked to the T869C variant and the G915C wild-type allele [127]. These polymorphisms have been explored in the context of HPV-related cancers, including SCCO. The TGFβ1 rs1982073 CT/CC variant alleles were shown to be strongly linked with better survival outcomes, including disease-specific and disease-free survival, in HPV16-positive SCCOP patients who had chemoradiation treatment, according to Tao et al. The rs1982073 polymorphism reduced the risk of death and recurrence, further highlighting its relevance in the clinical course of HPV16-positive SCCOP [128]. HPV is known to regulate the host DNA damage repair (DDR) pathway, promoting viral replication by modulating DNA repair mechanisms [129]. SMAD4, a key mediator of TGF-β signalling, is essential for preserving cell survival after genotoxic stress [121].

Citro et al. demonstrated that HPV-positive neck and head malignancies upregulate SMAD4, leading to increased expression of DDR proteins such as CHK1 and Rad51, which promote DNA repair and viral replication. Silencing SMAD4 in HPV-positive cells has been shown to increase sensitivity to cisplatin, suggesting that targeting SMAD4 may improve treatment efficacy in HPV-positive cancers [130].

Several GWAS have recognized risk loci for HNSCC, with the HLA locus being particularly notable. These studies suggest that genetic polymorphisms related to TGF-β1 and TGFβR1 contribute to variability in immune and inflammatory responses, which may influence susceptibility to HPV-positive OPSCC [131]. Niu et al. analyzed the correlation between TGFβ1 and TGFβR1 polymorphisms and treatment outcomes in HPV-positive OPSCC patients receiving chemoradiotherapy. Their findings underscore the importance of genetic polymorphisms in predicting treatment response and failure, with direct clinical implications for optimizing therapy [132]. TGFβ1 genotype has emerged as a predictive prognostic marker in HNSCC patients undergoing chemoradiotherapy, independent of p16 status [133]. Lundberg et al. confirmed that the TGFβ1 polymorphism is linked to improved disease-free survival rate in patients with HNSCC, independent of EGFR expression. These results prove that TGFβ1 might be a useful diagnostic indicator for HNSCC treatment [134] (Table 1)

Table 1. This table outlines therapeutic approaches targeting TGF-β in HPV-positive head and neck cancers, detailing therapies, mechanisms, biomarkers, and prognostic impacts in HNSCC.

Therapy Type	Target/Mechanism	Cancer Type	Biomarkers/Genes Involved	Prognosis	Reference
Immune Checkpoint Inhibitors	PD-1/PD-L1 blockade	HPV-positive HNSCC	PD-L1, TGF-β, CD4	Higher response rates in HPV-positive patients	[80]
Combination Therapy (Bintrafusp Alfa)	TGF-β trap and PD-L1 blockade	SCCHN, HPV-positive	TGF-β, PD-L1	33% response rate in HPV-positive tumors	[135]
Sorafenib + Cetuximab	EGFR and multi-kinase inhibition	Recurrent/Metastatic HNSCC	TGF-β, p16	Nosignificant effect; elevatedTGF-β associated with worse PFS	[136]

FcGBP Protein	Interaction with immune cells	HPV-positive HNSCC	FcGBP, TGF-β	Increased survival with high FcGBP and low TGF-β levels	[137]
miRNA Therapeutics	miR-9-5p targeting NOX4 and ROS	HPV-positive HNSCC	miR-9-5p, NOX4, TGF-β	Inhibition of TGF-β signalling, improved survival	[76]
TGF-β Inhibition + DNA Damage Repair	TGF-β inhibition	HPV-positive HNSCC	SMAD4, TGF-β, DNA repair	Enhanced radiosensitivity and chemotherapy response	[125]
Promoter Methylation Studies	Differential methylation	HPV-positive HNSCC	MSX2, TGF-β	Highlighted unique TGF-β signalling in HPV-positive cancers	[106]
TGF-β1 Polymorphisms	Polymorphisms affecting TGF-β1	HPV-positive SCCOP	TGF-β1, p16	Better survival with TGF-β1 rs1982073 CT/CC variant	[138]
Integrin alpha 6	Overexpression in CSCs	HPV-positive HNSCC	ITGα6, AKT	Poor prognosis with high ITGα6 expression	[139]
SMAD4 and DNA Repair	SMAD4 in DDR pathway	HPV-positive HNSCC	SMAD4, CHK1, Rad51	Enhanced DNA repair and resistance; low SMAD4 improves treatment	[140]
TGF-β1 and p16 Genotype	TGF-β1 polymorphisms	HPV-positive HNSCC	TGF-β1, p16	TGF-β1 polymorphisms linked to better outcomes	[134]

Future Directions And Perspectives

Developing more tailored therapeutics requires careful consideration of TGF-β's dual function in cancer, as a cancer suppressor in the initial stages and as an inducer of tumours in mature stages, according to the current consensus [141]. The focus is designing genetic and molecular approaches that selectively inhibit the tumor-promoting pathways of TGF-β while preserving its tumor-suppressive functions [142]. RNA interference (RNAi) technologies and CRISPR-Cas9 gene-editing methods are being developed to block key elements of TGF-β signalling contributing to cancer development [143]. Additionally, efforts are underway to create more potent and dual-specific TGF-β inhibitors with reduced toxicity [144]. Most noteworthy, there are bifunctional fusion proteins that simultaneously antagonize both TGF-β and PD-L1, and these seem to be planning for future preclinical and early clinical studies [142]. These strategies for targeting might mean inhibiting the tumour-promoting signals of TGF-β while restoring

antitumor immunity that was previously discussed, including immune checkpoint inhibitors [145]

The concepts of the new personalized medicine approaches promote incorporating TGF-β-targeting drugs into the anticancer therapy [146]. Tumors HPV-positive, for instance, should have different sensitivity to the treatment based on the targeting of TGF-β, since these types of cancer proved to have different immune contexts [9]. More targeted biomarkers, such as the TGF-β receptor expression levels or specific genetic mutations, may define the populations who can benefit from these drugs [147]. It also enables one to provide better therapy outcomes for the patient while at the same time avoiding more treatments, thus minimizing side effects. The improvements in biomarker-directed therapies are promising for the cancers because TGF-β is a crucial factor for tumour promotion [148].

Conclusion

TGF-β is a cytokine that has essential functions in contributing to damage and cellular migration regulation. Because of this, it is easier to design trials targeting the diverse role of FAK in tissue remodelling and tumorigenesis. Nevertheless, TGF-β inhibitors' anti-tumor effects are still unproven clinically owing to the lack of non-orientation and minimal use of these inhibitors in the clinical treatment of HNSCC. Promising outcomes in the HPV-positive population have been noted. Combinational strategies targeting TGF-β and common agents such as PD-L1 or EGFR inhibitors have provided uneven results. Several biomarkers have been identified to reflect the immune context as a key factor in determining the response to treatment indicated for advanced cancer, for instance, & no effort to implement them in clinical practice has been easy.

Due to the dual role of TGF-β, future therapeutic models must target the tumour-promoting action of TGF-β but spare its tumor suppressor actions. Further developing biomarkers and the new field of "-medicine'' will be paramount in creating an individualized therapy plan covering scientific aspects such as the patient's HPV status and TGF-B activity. The success of implementing TGF-β inhibition in clinical management will therefore depend on furthering these strategies and the characterization of the tumor microenvironment. Countering the complexities of the TGF-β signalling is critical in developing a better approach to cancer treatment especially for HPV-positive cancers, which are regulated by this cytokine.

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REVIEW ARTICALE

THERAPEUTIC POTENTIAL OF OCIMUM TENUIFLORUM PHYTOCHEMICALS IN ORAL CANCER-A COMPREHENSIVE REVIEW

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ABSTRACT

Background: Oral cancer remains a significant global health challenge, characterized by high mortality rates and limited effective treatment options. There is a growing interest in natural bioactive compounds as potential therapeutic agents to overcome the limitations of conventional therapies.

Purpose: This review aims to explore the therapeutic potential of bioactive compounds derived from *Ocimum tenuiflorum* L. (Holy basil or Tulsi) in the management of oral cancer.

Methods: A comprehensive literature survey was conducted PubMed, Scopus, and Web od Science database to analyze the mechanisms through which key phytochemicals from *O. tenuiflorum*, such as eugenol, rosmarinic acid, apigenin, and linalool, exert anticancer effects. Keywords included: "*Ocimum tenuiflorum*," "Tulsi," "Bioactive Compounds," "Nanotechnology," "Oral Cancer," "Personalized Medicine." We focused on modulating oxidative stress, inducing apoptosis, and inhibiting metastasis by interfering with critical signaling pathways.

Results: The selected phytochemicals demonstrated potent anticancer properties against oral cancer cells in preclinical studies. They effectively modulated oxidative stress, promoted apoptosis, and inhibited metastatic progression. However, significant gaps persist regarding their clinical applicability and long-term safety profiles.

Conclusions: *Ocimum tenuiflorum* exhibits transformative potential in oral cancer therapy. Future research should prioritize clinical translation of these preclinical findings and focus on developing novel therapeutic agents tailored to individual patient needs.

KEYWORDS: Bioactive Compounds, Nanotechnology, *Ocimum tenuiflorum*, Oral Cancer, Personalized Medicine

1. INTRODUCTION

Oral cancer, a subset of head and neck cancers, poses a significant global health burden, particularly in developing countries. It is characterized by the malignant transformation of epithelial cells in the oral cavity and is often diagnosed at advanced stages, contributing to high morbidity and mortality.[1]

Key etiological factors include tobacco use, alcohol consumption, poor oral hygiene, viral infections such as Human Papilloma Virus (HPV), and genetic predisposition. Despite the availability of conventional treatments such as surgery, chemotherapy, and radiation, these interventions often have limited efficacy, serious side effects, and high recurrence rates. Consequently, there is a growing interest in alternative and complementary therapies, particularly those based on medicinal plants, to

address these limitations. Among the many botanicals studied for their anticancer properties, *Ocimum tenuiflorum* L., commonly known as Holy basil or Tulsi, has emerged as a prominent candidate. Revered in Ayurvedic and other traditional systems of medicine, Tulsi is known for its wide spectrum of therapeutic properties. The plant contains a diverse array of phytochemicals, including eugenol, rosmarinic acid, apigenin, linalool, and other phenolic compounds, which have demonstrated potent antioxidant, anti-inflammatory, antimicrobial, and anticancer effects.[2,3,4,5] These compounds interact with multiple cellular pathways and have been shown to exert a significant influence on tumor growth and progression.

One of the primary mechanisms through which *O. tenuiflorum* exerts its anticancer effects is oxidative stress modulation. Oxidative stress plays a pivotal role in carcinogenesis by damaging cellular components and inducing mutations. The phytoconstituents in Tulsi exhibit strong free radical scavenging activities and enhance endogenous antioxidant defenses, thereby reducing the risk of cancer initiation and progression.[6] Additionally, Tulsi compounds can inhibit inflammatory pathways implicated in tumor promotion and metastasis, such as NF-κB and PI3K/Akt signaling. These pathways are essential for cancer cell survival, proliferation, and resistance to apoptosis. Another critical anticancer mechanism of *O. tenuiflorum* is the induction of apoptosis, or programmed cell death, in malignant cells. Compounds like apigenin and eugenol have been shown to modulate signaling pathways such as MAPK and HIF-1α, which regulate cell cycle arrest and apoptosis.[7,8] This selective cytotoxicity towards cancer cells, while sparing normal cells, presents a distinct advantage over conventional chemotherapy. Furthermore, *O. tenuiflorum* has been reported to inhibit angiogenesis, the process of new blood vessel formation that tumors rely on for growth and metastasis by downregulating pro-angiogenic factors such as VEGF.[1,9]

The affordability and accessibility of *O. tenuiflorum* make it an attractive option, especially in resource limited settings where oral cancer prevalence is disproportionately high. However, despite promising in vitro and in vivo findings, significant challenges remain. Variability in phytochemical composition due to environmental factors, limited bioavailability of active compounds, and lack of clinical validation are major obstacles to its integration into mainstream oncology.[10] Innovative approaches such as nanotechnology-based drug delivery systems have shown promise in enhancing the solubility, stability, and targeted delivery of Tulsi phytochemicals, potentially overcoming these limitations.[11] This review consolidates the current understanding of *O. tenuiflorum*'s phytochemical profile and its anticancer activities against oral malignancies. It also highlights emerging technologies and future directions aimed at optimizing its therapeutic potential. By bridging traditional wisdom and modern scientific research, *O. tenuiflorum* holds promise as a safe and effective adjunct in oral cancer management.

2. ORAL CANCER: EPIDEMIOLOGY AND RISK FACTORS

Oral cancer constitutes approximately 3% of all cancers worldwide, with a disproportionate burden in LMICs.[12,13,14] Regions such as South Asia, Eastern Europe, and parts of Latin America report some of the highest incidence rates, largely attributed to sociocultural habits like tobacco and areca nut use.[15] For example, India and Sri Lanka report alarmingly high prevalence of oral cancer due to widespread tobacco chewing and betel quid consumption. In contrast, Western countries show relatively lower incidence, though late-stage diagnoses and healthcare access inequalities persist.[16,17] The five-year survival rate remains suboptimal between 50–60% and is heavily dependent on the stage of diagnosis.[18] Patients diagnosed at earlier stages exhibit markedly better outcomes compared to those with advanced disease, highlighting the critical need for timely detection and treatment.[16,19] Multiple risk factors contribute to oral cancer development. Tobacco use and smoking, chewing, or using snuffs, remains the most significant carcinogen, delivering mutagenic agents that induce DNA damage and chronic inflammation.[20] Prolonged and intense tobacco use elevates cancer risk up to 15-fold.[15,21] Alcohol further exacerbates this risk, particularly when combined with tobacco. Metabolism of ethanol into acetaldehyde, a known carcinogen, disrupts DNA repair mechanisms, promoting malignancy.[22,23] In recent years, HPV, especially type 16, has emerged as a major risk factor for oropharyngeal cancer. Unlike tobacco-related cancers, HPV-positive cases often affect younger individuals and are localized to the tonsillar region and tongue base. The virus integrates into host DNA, leading to deregulation of oncogenes and inhibition of tumor suppressor proteins.[24,25]

Genetic predispositions, particularly mutations in DNA repair genes, can also elevate susceptibility to oral malignancies.[26,27] Environmental and dietary factors including high intake of smoked meats, refined carbohydrates, and nutrient-deficient diets have been implicated in modulating cancer risk.[28,29] Furthermore, recent studies implicate microbial dysbiosis in chronic oral inflammation and

carcinogenesis, pointing to a role for the oral microbiome.[30,31]

Early symptoms of oral cancer such as non-healing ulcers, leukoplakia, and unexplained lesions are often mistaken for benign conditions, resulting in delayed diagnosis and reduced therapeutic efficacy.[18,32] Existing diagnostic tools suffer from limitations in sensitivity and specificity, underscoring the urgent need for non-invasive biomarkers and improved screening strategies.[33,34]

Treatment typically involves surgery, often accompanied by radiotherapy and chemotherapy. While effective in managing localized disease, these approaches can result in debilitating side effects, functional impairment, and disfigurement.[35,36] Hence, there is a growing interest in alternative therapeutic strategies that are safer, more effective, and tailored to individual patient profiles.

3. TULSI: AN OVERVIEW

Tulsi (*O. tenuiflorum*) is a distinguished member of the Lamiaceae family, mostly used for its aromatic leaves and multipurpose applications in culinary, spiritual, and medicinal contexts. Native to the Indian subcontinent, this perennial herb thrives in warm, humid climates, growing up to 1 meter in height depending on environmental conditions.[37,38]

Its distinct elliptical, serrated leaves not only serve an ornamental role but also play a central part in traditional medical systems such as Ayurveda and Siddha.[39,40]

Tulsi holds profound cultural and religious significance in Hinduism, being revered as a sacred plant. Ancient scriptures detail its use in spiritual rituals, attributing purifying and protective properties to it.[41,42]

Beyond religious importance, *O. tenuiflorum* has been widely employed in managing ailments such as respiratory conditions, fevers, bronchitis, and digestive issues. Decoctions made from its leaves and stems have long served as remedies across traditional healthcare systems.[43]

The herb’s antimicrobial properties are particularly notable, with historical usage in wound healing, treating ulcers, and controlling oral inflammation and toothache in rural populations.[44,45] Classified as an adaptogen, *O. tenuiflorum* is known to bolster the body’s resilience to physical and emotional stress by regulating cortisol levels.[46]

Consumed regularly as teas or herbal supplements, it is integrated into health-promoting diets aiming to support holistic well-being.[47,48] Its therapeutic potential is attributed to a rich phytochemical profile, including phenolic acids, flavonoids, terpenoids,
and essential oils.[49,50] Compounds like rosmarinic acid and caffeic acid exhibit strong antioxidant activity by neutralizing free radicals, chelating metal ions, and reducing lipid peroxidation, thereby mitigating oxidative stress.[51] Flavonoids such as apigenin and orientin contribute anti-inflammatory and anticancer activities, making them promising agents in cancer research.[52,53]

The essential oils of Tulsi, rich in terpenoids like eugenol and linalool, contribute analgesic, antibacterial, and anticancer activities. Eugenol, in particular, has demonstrated the ability to inhibit tumor growth and modulate immune responses.[54,55] Additional oil components like citral and camphor, along with alkaloids, glycosides, and saponins, enhance its pharmacological efficacy.[56,57]

Multiple studies affirm the herb's safety, with consistent evidence showing minimal adverse effects at therapeutic doses in both animal and human trials.[58,59] Nonetheless, caution is advised for individuals with known hypersensitivity or those on certain medications. Pregnant and lactating women are advised to moderate intake due to insufficient safety data.[60,61] In preclinical disease models, *O. tenuiflorum* extracts have demonstrated anti-inflammatory effects through the suppression of pro-inflammatory cytokines and NF-κB signaling pathways.[62,63]

Its anticancer potential is evidenced by its ability to inhibit tumor cell proliferation, induce apoptosis, and suppress metastasis, primarily through bioactive compounds like eugenol and rosmarinic acid, which regulate MMPs and VEGF, respectively.[64,65]

Advanced extraction techniques such as supercritical fluid extraction and ultrasound-assisted methods are being adopted to optimize the purity and potency of Tulsi based formulations.[66] These advancements bolster its value in modern integrative medicine and reaffirm its relevance in both traditional and contemporary healthcare systems.

4. BIOACTIVE COMPOUNDS IN TULSI

Tulsi contains multiple bioactive compounds, notably eugenol, rosmarinic acid, apigenin, and linalool, each contributing to its remarkable anticancer properties, especially in oral cancer. (**Figure 1, Table 1**).

Table 1. Anticancer properties of major bioactive compounds from *O. tenuiflorum* (Tulsi)

Compound	Key Mechanisms	Molecular Targets / Pathways	Cancer-Relevant Effects	References
Eugenol	Antioxidant, pro-apoptotic, anti-inflammatory, anti-metastatic	ROS scavenging, ↑SOD & catalase, ↓PI3K/Akt/mTOR, ↓NF-κB, ↓MMP-2/9, ↑cytochrome c	Induces apoptosis, inhibits proliferation, EMT, metastasis, and enhances chemosensitivity	38,67,68
Rosmarinic Acid	Antioxidant, anti-inflammatory, pro-apoptotic	↓IL-6, IL-8, TNF-α, ↓NF-κB, ↓STAT3, ↑Bax, ↓Bcl-2	Enhances intrinsic apoptosis, modulates tumor microenvironment, synergizes with eugenol	46,56,57
Apigenin	Cell cycle arrest, pro-apoptotic, anti-angiogenic	↓CDK2/4, ↑p21/p27, ↑caspase cascade, ↓VEGF	Induces G1/S arrest, promotes apoptosis, inhibits angiogenesis	60,69
Linalool	Antioxidant, mitochondrial apoptosis, anti-inflammatory	↓ROS, ↑SOD, ↓IL-6, ↓VEGF, ↓MMPs	Induces apoptosis, inhibits metastasis, angiogenesis	46,70,71
Synergistic Action	Multi-targeted effect	Combination of above pathways	Enhances therapeutic response, overcomes drug resistance, minimal cytotoxicity to normal cells	72,73

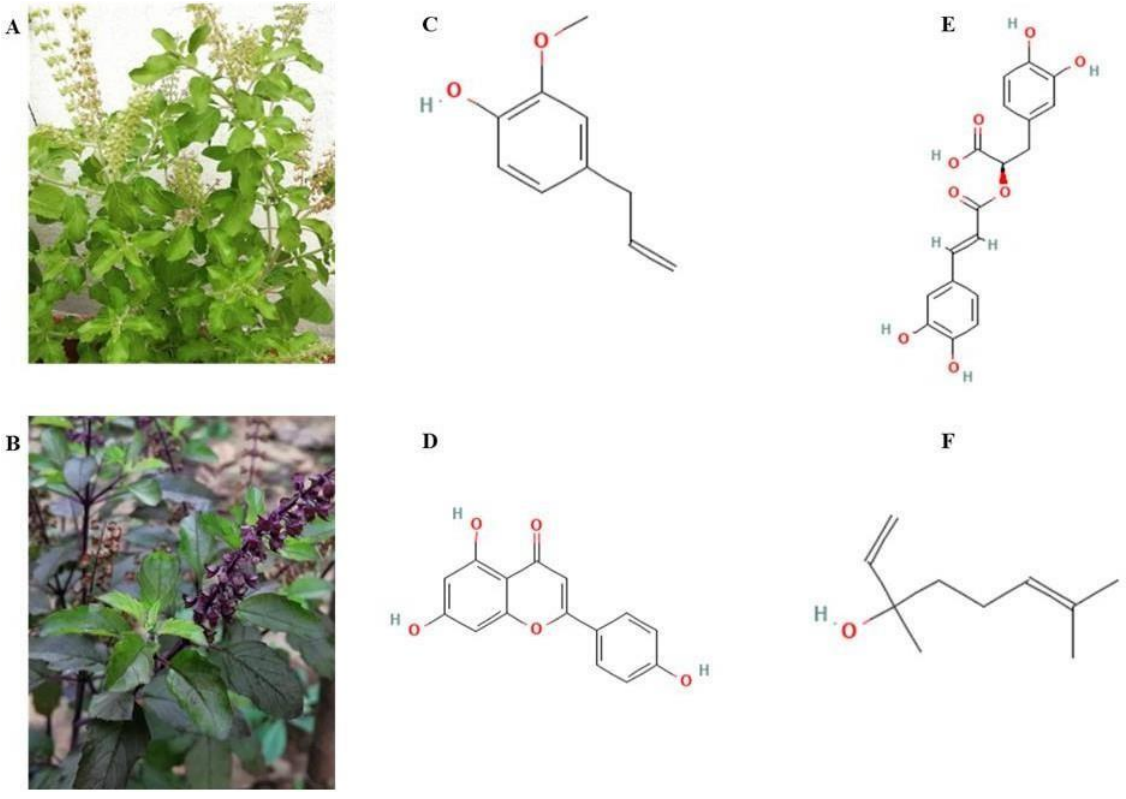


Figure 1. Key constituents of *O. tenuiflorum* with anticancer potential. (A) *O. tenuiflorum* (Green type); (B) *O. tenuiflorum* (Purple type); (C) Eugenol; (D) Apigenin; (E) Rosmarinic acid; (F) Linalool. These compounds contribute to oxidative stress modulation, apoptosis induction, metastasis inhibition, and oncogenic pathway regulation in oral cancer.

Eugenol, a phenylpropanoid, is the most extensively studied compound from *O. tenuiflorum*. It permeates cell membranes and targets mitochondrial pathways, disrupting oxidative stress balance and inducing apoptosis. Eugenol scavenges reactive oxygen species (ROS) and enhances antioxidant enzymes such as superoxide dismutase (SOD) and catalase, thus reducing oxidative stress implicated in carcinogenesis.[74,75] It also impairs the electron transport chain, promoting cytochrome c release and activating the intrinsic apoptotic pathway.[46]

Eugenol downregulates anti-apoptotic proteins (e.g., Bcl-2) while upregulating pro-apoptotic proteins (e.g., Bax), shifting the balance toward cell death.[73] Eugenol modulates critical oncogenic pathways like PI3K/Akt/mTOR and NF-κB. It inhibits Akt phosphorylation, reducing glucose uptake and halting tumor growth.[38] By suppressing NF-κB, it lowers inflammation and anti-apoptotic gene expression, both of which are central to cancer progression.[43] Eugenol also exhibits anti-metastatic properties by downregulating matrix metalloproteinases MMP-2 and MMP-9 and reversing epithelial-to-mesenchymal transition (EMT), enhancing E-cadherin while suppressing vimentin.[71] Particularly, eugenol enhances the efficacy of chemotherapeutics like cisplatin by inhibiting multidrug resistance (MDR) proteins, thereby increasing intracellular drug accumulation. Its selective cytotoxicity toward cancer cells, attributed to their altered redox states, and its lipophilic properties, enabling better mucosal penetration, make it ideal for oral cancer therapy.[56]

Rosmarinic acid, a polyphenol found in *O. tenuiflorum*, complements eugenol through its potent anti-inflammatory and antioxidant properties. It inhibits pro-inflammatory cytokines such as IL-6 and TNF-α, altering the tumor microenvironment.[76] At the molecular level, it suppresses STAT3 and NF-κB signaling pathways, reducing cancer cell proliferation and enhancing apoptosis.[57] Additionally, it modulates Bcl-2 family proteins, increasing Bax/Bak and decreasing Bcl-2 expression, leading to mitochondrial membrane destabilization and caspase activation. It also arrests the cell cycle at the G1/S checkpoint by downregulating cyclins and CDKs. [70]

Apigenin, a flavonoid in *O. tenuiflorum*, induces cell cycle arrest and apoptosis. It inhibits CDK2/CDK4 activity, halting the G1/S transition, and upregulates p21 and p27 to reinforce checkpoint control.[69] Like eugenol and rosmarinic acid, apigenin promotes cytochrome c release and caspase cascade activation. It also suppresses inflammatory mediators and reduces VEGF expression, impairing angiogenesis.[60]

Linalool, a monoterpene, exerts antioxidant and anti-inflammatory effects by scavenging ROS and downregulating cytokines, thereby reducing tumor-favoring inflammation.[70] It induces mitochondrial depolarization, cytochrome-c release, and apoptosis. Like other compounds in *O. tenuiflorum*, it inhibits MMPs and VEGF, limiting metastasis and angiogenesis.[46] The synergistic interactions among these phytochemicals offer a holistic and multi-targeted strategy against cancer. Their collective activities apoptotic induction, oxidative stress modulation, signaling pathway disruption, and metastasis inhibition highlight the therapeutic promise of *O. tenuiflorum*. Ongoing research continues to explore these compounds for future applications in integrative and localized oral cancer therapy.

5. MECHANISMS OF ACTION AGAINST ORAL CANCER

The bioactive compounds of *O. tenuiflorum*, notably eugenol, rosmarinic acid, and apigenin, exhibit promising chemo-preventive and therapeutic potential against oral cancer. These phytochemicals exert synergistic effects across multiple pathways, targeting oxidative stress, inflammation, cellular signaling, apoptosis, and metastasis, while sparing normal cells (**Figure 2**). Oxidative stress and chronic inflammation are critical contributors to oral carcinogenesis. Excessive reactive oxygen species (ROS) promote DNA damage, genomic instability, and tumor progression.[77] Eugenol, the major active component of *O. tenuiflorum*, demonstrates potent antioxidant activity by scavenging superoxide anions and hydroxyl radicals.[78,79]

It also enhances endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, thereby restoring redox homeostasis and reducing oxidative DNA damage.[78,80] Inflammation is another hallmark of cancer progression. Elevated levels of cytokines like IL-6 and TNF-α promote a pro-tumorigenic microenvironment.[77]

Rosmarinic acid and linalool, present in *O. tenuiflorum*, modulate inflammation by downregulating IL-6, IL-8, and TNF-α expression and inhibiting the NF-κB pathway, a central mediator of inflammation.[77,81]

This downregulation mitigates chronic inflammation and contributes to tissue homeostasis. Disruption of oncogenic signaling pathways further strengthens the anticancer efficacy of these phytocompounds. The PI3K/Akt/mTOR signaling axis, commonly activated in oral cancers, promotes proliferation and apoptosis resistance.[82] Eugenol and apigenin inhibit Akt phosphorylation, leading to downstream suppression of mTOR signaling and halting cell cycle progression.[81,83] Additionally, suppression of NF-κB phosphorylation and nuclear translocation by eugenol and rosmarinic acid blocks the transcription of anti-apoptotic and pro-inflammatory genes, sensitizing cancer cells to cell death.[84,85]

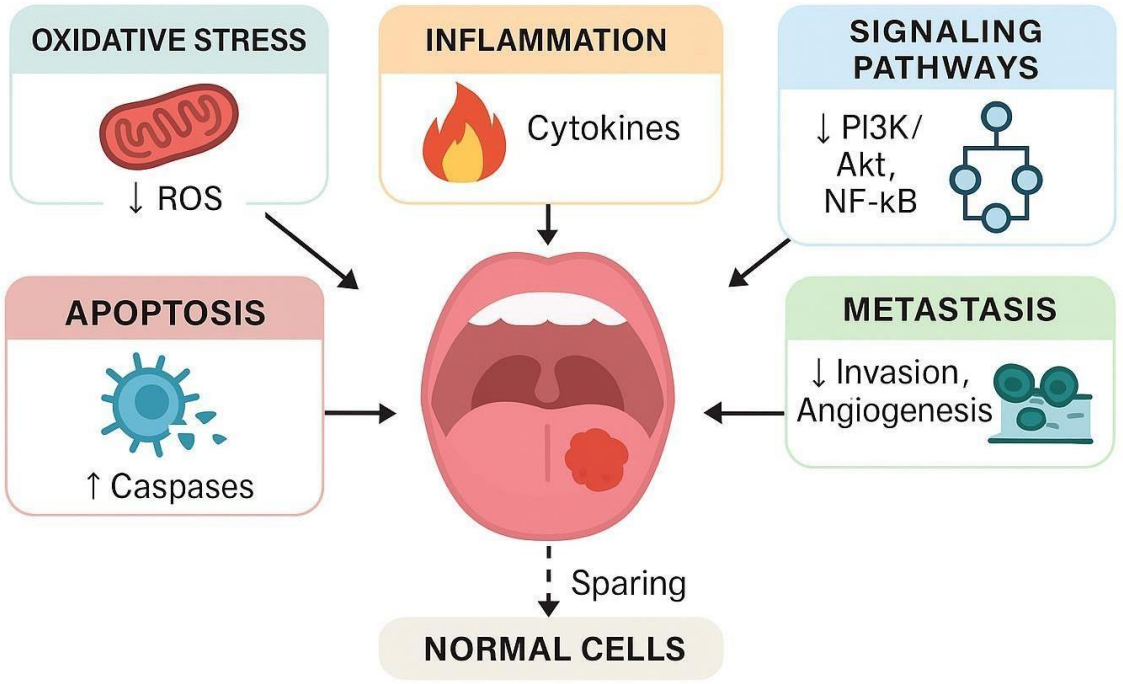


Figure 2. Mechanisms of *Ocimum tenuiflorum* bioactive compounds against oral cancer, including modulation of oxidative stress, suppression of inflammation, inhibition of PI3K/Akt and NF-κB signaling, induction of apoptosis, and prevention of metastasis and angiogenesis, while sparing normal cells.

Apoptosis induction through the intrinsic mitochondrial pathway is a pivotal mechanism. Eugenol disrupts mitochondrial membrane potential, triggering cytochrome c release and activating caspase-9 and caspase-3, culminating in apoptosis.[86,87] Apigenin complements this effect by upregulating pro-apoptotic proteins and downregulating anti-apoptotic Bcl-2, thus promoting selective apoptosis of cancer cells.[88,89] Cell cycle arrest is another key target. Apigenin inhibits G1/S phase transition by downregulating cyclin D1 and CDK4 while upregulating p21, a cyclin-dependent kinase inhibitor.[90,91] This blockade prevents uncontrolled cell proliferation and facilitates apoptotic signaling. The inhibition of metastasis and angiogenesis by *O. tenuiflorum* compounds further enhances its anticancer potential. Eugenol and linalool suppress matrix metalloproteinases MMP-2 and MMP-9, enzymes essential for extracellular matrix degradation and cancer cell invasion.[92,93] Eugenol also represses epithelial-to-mesenchymal transition (EMT) by restoring E-cadherin expression and reducing vimentin levels, thus reducing metastatic spread.[94,95] Angiogenesis, a process vital for tumor survival and expansion, is impeded by apigenin and rosmarinic acid, which inhibit vascular endothelial growth factor (VEGF) expression.[96,97,98] The inhibition of VEGF limits endothelial cell recruitment and neovascularization, depriving tumors of essential nutrients and oxygen. Collectively, these bioactive compounds act on diverse molecular targets to combat oral cancer. Their multi-modal mechanisms as antioxidant, anti-inflammatory, anti-proliferative, pro-apoptotic, anti-metastatic, and anti-angiogenic position, Tulsi as a potent natural adjunct in oral cancer therapy, warranting further exploration in clinical settings.

6. PRECLINICAL STUDIES ON *O. TENUIFLORUM* AND ORAL CANCER

Preclinical investigations provide strong evidence for the anticancer efficacy of *O. tenuiflorum* in oral cancer models (**Table 2**). These studies, involving both in vitro assays using oral cancer cell lines (e.g., SCC-9, SCC-25, CAL-27) and in vivo animal models, highlight the therapeutic potential of its bioactive compound's eugenol, rosmarinic acid, apigenin, and linalool. Eugenol, a dominant phytochemical in *O. tenuiflorum*, exhibits potent cytotoxic effects by inhibiting cancer cell proliferation and inducing apoptosis in a dose-dependent manner. The apoptotic effects are marked by morphological changes such as cell shrinkage and nuclear condensation.[38,99] Mechanistically, eugenol disrupts mitochondrial membrane potential, triggering cytochrome c release, and activation of caspase-9 and caspase-3, thereby initiating the intrinsic apoptotic pathway.[43] Importantly, eugenol demonstrates selective toxicity, preferentially targeting cancer cells while sparing normal cells, which enhances its therapeutic appeal.

Table 2. Preclinical Evidence Supporting the Anticancer Potential of *O. tenuiflorum* in Oral Cancer

Bioactive Compound	Experimental Model	Mechanisms of Action	Key Outcomes in Oral Cancer Models	References
Eugenol	*In vitro* (SCC-9, SCC-25, CAL-27); *In vivo* (4-NQO, DMBA models)	↓Mitochondrial membrane potential, ↑cytochrome c, ↑caspase-9/3, ↓Bcl-2, ↑Bax	Apoptosis induction, selective cytotoxicity, reduced lesion severity, no organ toxicity	38,43,99
Rosmarinic Acid	*In vitro*; *In vivo* (rat oral carcinogenesis models)	G1/S arrest, ↓Cyclin D1, ↓CDK4, ↑p21, ↓IL-6, TNF-α, ↑antioxidants	Inhibits proliferation, suppresses inflammation, enhances DNA protection	71,100
Apigenin	*In vitro* oral cancer cell lines	↑p21/p27, ↓Cyclin D1/CDK4, ↑Caspase-3, ↓Bcl-2	Cell cycle arrest, apoptosis promotion, synergy with eugenol/rosmarinic acid	71,89,101
Linalool	*In vitro* and *in vivo* models	↓IL-6, ↓TNF-α, ↓MMP-2/9, ↓NF-κB, ↓migration/invasion	Anti-inflammatory, anti-metastatic, inhibits EMT	43,102,103
Whole Extract (O. tenuiflorum)	Rodent carcinogen-induced oral cancer models (4-NQO, DMBA)	↑SOD, ↑catalase, ↓ROS, ↓hyperplasia/dysplasia	Reduction in tumor incidence and lesion grade; preserves liver/kidney health	71,104,100
Combination with Chemotherapeutics	Eugenol + Cisplatin / Rosmarinic Acid + Doxorubicin	↓MDR proteins, ↑chemosensitivity, ↓toxicity	Synergistic effects; enhanced efficacy and tolerability vs. standard chemotherapy alone	38,93,105

Rosmarinic acid further contributes to anticancer activity by inducing cell cycle arrest at the G1/S checkpoint through downregulation of cyclin D1 and CDKs, and upregulation of the CDK inhibitor p21.[71,100] It also cooperates with eugenol in modulating apoptosis-related proteins, upregulating Bax and downregulating Bcl-2.[60] These synergistic actions enhance the overall anticancer potency of *O. tenuiflorum* extracts. Apigenin, another bioactive flavonoid in *O. tenuiflorum*, complements these effects by reinforcing intrinsic apoptosis in oral cancer cells, amplifying the impact of eugenol and rosmarinic acid.[71,101] Collectively, these compounds present opportunities for combination therapy with enhanced efficacy and lower toxicity.[43] Linalool, a monoterpenoid component, provides anti-inflammatory and antimetastatic effects. It suppresses pro-inflammatory cytokines such as IL-6 and TNF-α and inhibits matrix metalloproteinases (MMPs), reducing tumor-promoting inflammation and cell invasion.[102,103] These effects underscore *O. tenuiflorum* role in preventing metastasis and promoting cancer cell containment.

The *in vivo* studies using rodent models with chemically induced oral cancer (e.g., 4-NQO and DMBA) validate the chemo-preventive effects of *O. tenuiflorum* extracts. Treatment results in reduced incidence and severity of precancerous lesions and histological improvements, including decreased dysplasia and hyperplasia.[71,101] These outcomes are linked to enhanced antioxidant defenses and reduced oxidative stress via free radical scavenging by eugenol and rosmarinic acid.[103,104]

Additional mechanistic insights show that pyrazine derivatives and linalool modulate pro-apoptotic signaling and downregulate inflammatory markers, improving organ health and supporting the systemic safety of the extract.[106,107,108] Toxicological evaluations confirm that therapeutic doses of *O. tenuiflorum* do not adversely affect the liver, heart, or kidneys, validating its biocompatibility.[99,104] Comparative analyses with chemotherapeutic agents reveal that eugenol matches or surpasses cisplatin in cytotoxic activity, while causing less damage to healthy tissues.[38] Moreover, rosmarinic acid and apigenin can enhance doxorubicin's effect by mitigating multidrug resistance mechanisms.[93,105] Co-administration of *O. tenuiflorum* with chemotherapeutics enhances overall cytotoxicity and reduces required dosages, thereby minimizing side effects and improving patient tolerance.[71] Overall, preclinical data firmly support the chemo-preventive and therapeutic potential of *O. tenuiflorum* in oral cancer. Its multi-targeted mechanisms, safety profile, and synergy with existing drugs make it a strong candidate for future clinical trials in oncology.

7. DRUG DELIVERY SYSTEMS FOR ENHANCING BIOAVAILABILITY

The therapeutic potential of *O. tenuiflorum* in treating oral cancer is limited by challenges in the bioavailability and stability of its bioactive compounds such as eugenol, rosmarinic acid, and apigenin. These phytochemicals possess low aqueous solubility, high lipophilicity, and are prone to rapid metabolic degradation, limiting their systemic absorption and clinical efficacy.[28,109,110] Due to their lipophilic nature, compounds like eugenol and apigenin are poorly absorbed in conventional oral formulations, reducing their therapeutic concentrations in target tissues.[111,112] In addition to poor solubility, many of these constituents undergo rapid enzymatic metabolism primarily via cytochrome P450 enzymes resulting in the formation of less active metabolites before they reach systemic circulation.[112] For example, rosmarinic acid is especially unstable when exposed to environmental stressors like pH fluctuations, light, and heat, requiring special formulation strategies to preserve its bioactivity.[111,113] These pharmacokinetic drawbacks result in subtherapeutic plasma levels that are inadequate for meaningful anti-cancer activity. Furthermore, the non-targeted nature of traditional delivery systems increases the risk of off-target effects. When administered orally or topically, these bioactives are dispersed non-specifically throughout the body, reducing their concentration at cancer sites and increasing the risk of side effects in healthy tissues.[114,115] This lack of site-specificity is a major limitation in cancer therapy, where precise drug delivery is essential.

Advancements in nanotechnology have introduced novel drug delivery systems that address these limitations. Nanocarriers such as liposomes, polymeric nanoparticles, and solid lipid nanoparticles (SLNs) can encapsulate lipophilic phytochemicals, enhancing solubility, stability, and targeted delivery.[116,117,118] Liposomes protect compounds from degradation and improve membrane permeability. Functionalization with tumor-specific ligands enables targeted delivery, increasing therapeutic efficacy and minimizing systemic toxicity.[109,119,120,121] Polymeric nanoparticles made from biocompatible materials like chitosan and polylactic-co-glycolic acid (PLGA) offer controlled, sustained release of bioactives. PLGA-based systems have shown to enhance the bioavailability of rosmarinic acid, boosting cytotoxicity against tumor cells while protecting normal tissues.[122,123,124] SLNs provide a hybrid approach, stabilizing lipophilic compounds within a solid lipid matrix, improving pharmacological properties, and allowing for stimulus-responsive, localized release at tumor sites.[118,125,126] Combination therapies incorporating phytochemicals with conventional chemotherapeutics have shown synergistic effects, reducing the required doses of synthetic drugs and associated side effects.[127,128,129] Solubility enhancers like cyclodextrins and hydrocolloid carriers have also been effective in improving the pharmacokinetics of poorly soluble compounds.[130,131] While natural product-based therapies are attractive due to their low toxicity and rich pharmacological profiles, their success depends on innovative delivery systems that address inherent limitations in solubility, metabolism, and targeting.[132,133] Integrated therapeutic strategies combining nanocarriers and natural phytochemicals may be especially effective in complex conditions like oral cancer.[124,134] *O. tenuiflorum* holds immense potential for oral cancer treatment, but overcoming its bioavailability hurdles is essential. Advanced drug delivery platforms, particularly those leveraging nanotechnology, offer promising avenues to maximize its therapeutic value. Further *in vivo* and clinical validations are warranted to translate these promising results into effective cancer therapeutics.

8. INTEGRATION WITH CONVENTIONAL THERAPIES

The integration of *O. tenuiflorum* compounds with conventional cancer therapies has gained prominence for enhancing therapeutic efficacy and minimizing side effects from chemotherapy and

radiotherapy. This synergistic approach involves combining natural bioactive compounds from *O. tenuiflorum* with standard treatments to optimize outcomes tailored to individual patients. Eugenol, a major constituent of *O. tenuiflorum*, has shown promise in potentiating the effects of chemotherapeutics such as cisplatin. Preclinical studies indicate that the co-administration of eugenol with cisplatin significantly reduces tumor volume compared to cisplatin alone.[135] Eugenol's mechanism includes downregulation of multidrug resistance (MDR) proteins like P-glycoprotein (P-gp), thereby enhancing intracellular drug retention and efficacy.[136,137]

Additionally, its antioxidant properties may reduce cisplatin-induced nephrotoxicity, preserving renal function.[138] Other *O. tenuiflorum* compounds also contribute to combination therapy. Rosmarinic acid, for example, augments the cytotoxic effects of doxorubicin by inducing apoptosis and cell cycle arrest.[70] Linalool, another bioactive component, acts as a radiosensitizer, increasing radiation-induced cytotoxicity by elevating reactive oxygen species (ROS) and inhibiting DNA repair in tumor cells.[135,139,140] This dual effect enhances tumor cell mortality while potentially protecting normal tissues via antioxidant mechanisms.[141]

The rise of personalized medicine further advances the clinical utility of *O. tenuiflorum*. Tailored treatment regimens based on genetic polymorphisms in drug metabolism can optimize dosages and therapeutic response.[136,142] Proteomic and metabolomic profiling aids in identifying biomarkers predictive of responsiveness to *O. tenuiflorum*, supporting stratified treatment.[143] Additionally, combining eugenol with targeted therapies may offer enhanced outcomes in patients with mutations in specific signaling pathways.[144] Thus, incorporating *O. tenuiflorum* compounds into standard and personalized cancer treatments offers a promising strategy to boost efficacy and reduce toxicity. Continued research may solidify their role in integrative oncology.

9. CLINICAL RELEVANCE AND FUTURE DIRECTIONS

Compounds from *O. tenuiflorum* show significant potential in oral cancer therapeutics. Bioactive compounds like eugenol, linalool, and rosmarinic acid demonstrate antioxidant, anti-inflammatory, and chemoprotective effects, making them valuable for both prevention and treatment.[38,145] For instance, eugenol has been shown to reduce cisplatin-induced nephrotoxicity and potentially enhance its efficacy when co-administered.[38,45] Linalool, with radiosensitizing properties, may enhance radiation therapy effectiveness while reducing collateral tissue damage.[51,146]

The chemo-preventive role of *O. tenuiflorum* is compelling, particularly for individuals at risk due to tobacco, alcohol, or HPV exposure. Rosmarinic acid and apigenin may counteract chronic inflammation and oxidative stress, pivotal in oral cancer development.[147,148,149]

Additionally, localized applications, such as mouthwashes or lozenges, show promise for addressing precancerous lesions.[45,150] Challenges include standardizing extracts, considering variations in phytochemical composition.[75] Further exploration of pharmacokinetics and potential drug interactions, particularly with cytochrome P450 enzymes, is essential to ensure safety and efficacy.[151,152] Understanding molecular mechanisms via pathways like PI3K/Akt/mTOR and NF-κB is critical for optimizing therapeutic regimens.[153,154] Advancements in medicinal chemistry, nanotechnology, and theranostic platforms could revolutionize *O. tenuiflorum* clinical application.[155,156] Investigating its potential in combinatorial therapies, particularly with immunotherapy, could further enhance treatment outcomes.[45,157]

10. CONCLUSION

O. tenuiflorum, with its bioactive compounds such as eugenol, rosmarinic acid, apigenin, and linalool, offers a transformative approach to oral cancer treatment by targeting key cancer hallmarks like oxidative stress, inflammation, apoptosis, and metastasis. Preclinical studies demonstrate their potential in reducing tumor growth, enhancing the effects of conventional therapies, and reducing chemotherapy and radiation related toxicities. Nanotechnology-based drug delivery systems have helped overcome bioavailability challenges, ensuring efficient targeting of affected tissues. The synergy between *O. tenuiflorum* and conventional therapies highlights its potential to improve therapeutic outcomes while minimizing side effects. Personalized medicine approaches, utilizing genetic, proteomic, and metabolomic data, could further tailor treatments to individual patients, optimizing safety and efficacy. However, challenges remain, including the need for standardized extracts, long-term toxicity studies, and a deeper understanding of its mechanisms to enable clinical application. The development of novel therapeutic agents through synthetic modifications or advanced platforms, such as theragnostic nanoparticles, could enhance its impact. Beyond its anticancer potential, Tulsi shows promise in chemoprevention, palliative care, and integrative oncology, addressing both physical and psychosocial aspects of cancer management. As research

progresses, *O. tenuiflorum* holds great promise in revolutionizing oral cancer treatment, providing safer, more effective, and personalized solutions. This botanical powerhouse exemplifies the intersection of nature and technology, offering hope for better patient outcomes and a future where oral cancer can be managed with precision and compassion.

DECLARATIONS

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CONFLICT OF INTEREST

Authors declared that they do not have any conflicts of interest.

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ORIGINAL RESEARCH

MUSCULAR-APONEUROTIC SYSTEM OF THE SKIN OF THE FACE, AS A UNIQUE STRUCTURE OF THE FACIAL SCULL (CRANIUM FACIALIS) OF HIGHER ANIMALS

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ABSTRACT

Aim: The purpose of this research was to study the organization of collagen in animal skin in order to find similar SMAS structures.

Materials and Methods: The studies were conducted on skin samples from sheep and pigs (from the facial area), as well as trout. The obtained samples were studied under ultraviolet radiation. The Qscan Plus (AIOBIO, South Korea) device with a wavelength of 405 nm was used as a source of ultraviolet radiation. Skin sections were made at right and oblique angles in order to establish the spatial configuration of collagen fibers. The density (concentration) of fibers per unit area was calculated qualitatively by micro- and macroscopic methods with photometry. The results were evaluated in the Microsoft Office software package.

Results: In all the studied facial skin samples, the presence of collagen luminescent white in ultraviolet light was detected. The collagen was located laminarly, parallel to the skin surface, without the formation of a three-dimensional mesh structure characteristic of the SMAS of human facial skin. Conclusions: The data confirm the presence of collagen in the facial skin of the studied animals. However, unlike human facial skin, the characteristic three-dimensional collagen organization of the SMAS has not been detected. Further research is needed to determine the evolutionary origin of the SMAS and identify the factors that determine its formation.

Conclusion: No structures similar to SMAS were found in the animals used in the experiment. This is most likely due to the function performed by SMAS – the transmission of facial contractions to the skin. Probably, the structural organization of collagen on the human face is unique. Further research is required to accurately determine the evolutionary development of SMAS.

Key words: SMAS; facial skin; collagen; ultraviolet radiation.

1. INTRODUCTION

Collagen is a fibrillar protein that is the main component of the extracellular matrix and provides structural support and tissue function. The diversity of types, properties, and wide distribution of this protein make it valuable for both scientific research and various industrial applications (cosmetology, medicine, food industry).[1] Differences in the biomechanical characteristics of collagen obtained from the skin of different animals (fish, pigs, sheep) make it important to study its origin and organization. Also, the structure and quantity of collagen are important diagnostic indicators.

Fibrillar collagen is the most abundant collagen in the body. Collagens types I, II, and III account for at least 80–90% of all collagen in the body.[2] The fundamental subunit of a collagen fibril is called tropocollagen. It is a protein molecule 1.5 nm in diameter and 300 nm in length, consisting of three polypeptide α-chains organized into a triple helix.[3] The triple helix of type I collagen molecules (the most common type) consists of polypeptide chains, each of which contains a repeating sequence GXY, where G is glycine, and X and Y usually correspond to proline or hydroxyproline. The diameter of collagen fibrils ranges from 50 to 200 nm. When arranged together, fibrils form fibrous bundles with a diameter of 500 to 3000 nm.[4,5] The three-dimensional structure of the collagen network varies significantly depending on its location in the body and its biological purpose. For example, collagen fibers in the arterial wall are densely packed and oriented along the length of the vessel.[6]

It is clear from the literature that collagen as a structural unit is present in many animals, especially in marine fish [7, 8], making them a natural source of additional nutrition for individuals with deficiencies. Non-invasive methods based on the use of ultraviolet radiation with a wavelength of 405 nm allow for the effective diagnosis of oral diseases and assessment of tissue quality due to their high sensitivity. Moreover, in the facial skin of humans and monkeys, collagen is organized into a unique three-dimensional structure – SMAS. The evolutionary origin of SMAS remains a subject of research.

It is evident from the literature that collagen as a structural unit is present in many animals, especially in marine fish [7, 8], making them a natural source of additional nutrition for individuals with deficiencies. The aim of study is to compare the organization of collagen in the facial skin of different animal species (fish, pigs, sheep) and humans to find structural analogs of SMAS and to clarify its evolutionary history. Our preliminary data confirm the presence of collagen in facial skin samples from all animals studied, but the characteristic three-dimensional organization inherent to SMAS has not been identified.

2. MATERIAL AND METHODS

The studies were conducted on biological material: ram (n=10), pig (n=10), trout (n=10). Freshly obtained material was used in the work, storage conditions were natural cold without preliminary embalming. The skin material of the ram and pig was collected from the facial area of the skull. The obtained samples were immediately studied under ultraviolet radiation. The Qscan Plus device (AIOBIO, South Korea) with a wavelength of 405 nm was used as a source of ultraviolet radiation. Skin sections were made at right and oblique angles in order to establish the spatial configuration of collagen fibers. The density (concentration) of fibers per unit area was calculated qualitatively by micro- and macroscopic methods with photometry.

The solubility of acid-soluble and pepsin-soluble collagens was studied. The results were evaluated in the Microsoft Office software package.

3. RESULTS

The skin samples of sheep, trout and pig were examined. The macrophotographic results obtained were photographed and presented in figures 1 – 3.

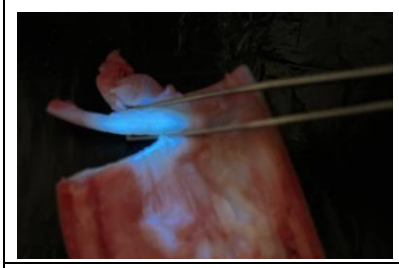


Figure 1. Cross-section of pig skin under ultraviolet light

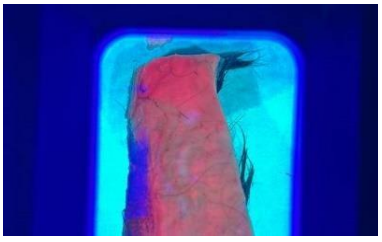


Figure 2. Ram skin under ultraviolet light



Figure 3. Trout skin under ultraviolet light

The presence of collagen was detected in all samples. Collagen is known to fluoresce white under ultraviolet light, which was detected. Arrows in the figures indicate areas where collagen accumulation was detected. In the studied samples, the highest collagen content was detected in trout (Table 1).

Table 1. Collagen content in the samples

Sample number and its characteristics	Sample 1 (n=10)	Sample 2 (n=10)	Sample 3 (n=10)
Density of collagen fibers in the superficial layers	low	low	high
Collagen fiber density	high	low	high
Skin flap thickness in mm	2,5±0,2	1,4±0,1	0,6±0,1

In all three samples, collagen was arranged linearly, laminarly, without forming a three-dimensional structure. This fact was confirmed by performing several tissue sections in different places. The direction of the collagen fibers was parallel to the skin surface, and no mesh structure was observed.

The study examined the solubility of acid-soluble and pepsin-soluble collagens isolated from pig, sheep and trout skin, depending on the pH level. The results are presented below.:

1. High solubility in acidic environments (pH 1–4):
   - Collagens from all three species (porcine, sheep, and trout) demonstrated high solubility in the pH range of 1–4.
   - Maximum solubility was observed at pH 3.
   - Acid-soluble collagen: 90.2 ± 1.5%.
   - Pepsin-soluble collagen: 93.4 ± 2.1%. This indicates that collagens are largely soluble in strong acid.

2. Decreased solubility in a neutral environment (pH 4–6):

o The solubility of both collagen types decreased sharply in the pH range from 4 to 6.

o The minimum solubility was observed at pH 6:
   - Acid-soluble collagen: 35.2 ± 3.1%
   - Pepsin-soluble collagen: 38.4 ± 3.3%

DISCUSSION

It is known that collagen is present in the skin, connective tissue and many other structures of various animals. Evolutionarily, it is not entirely clear in which animals collagen first appeared. It is known that proteins of the collagen family are found in sponges (Porifera) - animals phylogenetically distant from humans.[9]

Animals are a classic source of collagen in industry, which is used for cosmetic procedures, wound treatment, stopping bleeding, and as a food additive.[10 - 12] Collagen is most often obtained from the skin of fish and pigs, sheep are also used for this purpose, and the structure and properties of collagen vary depending on the source of its production.

Thus, pig collagen has better mechanical properties than collagen based on fish skin.[11]

Collagen studies using ultraviolet radiation are gaining popularity due to their low invasiveness and high visibility of the results. Radiation with a wavelength of 405 nm is proposed by researchers for the early diagnosis of oncological diseases of the oral cavity. In this case, areas lacking collagen appear dark under the radiation, and areas with a normal collagen content appear lighter.[14] Also, the phenomenon of collagen autofluorescence can be used to assess the biological properties of tissue transplants.[15]

Despite the fact that the evolutionary predecessors of humans have collagen in the skin of the facial area, its structural organization differs from that of humans. It has been found that collagen on human facial skin is part of the SMAS - the Superficial Muscle-Aponeurotic System. This structure was first described in 1976 by researchers Mitz and Peyronie.[16] Since then, several types of SMAS have been identified, differing in structure and location on the face. Using 3D reconstruction technologies, a 3D model of SMAS was constructed based on histological preparations. In the course of such a study, it was discovered that SMAS is not a chaotically arranged collagen fibers, but a three-dimensional network that forms interconnected spaces.

The fibrous septa of SMAS form communicating compartments filled with adipose tissue.[17, 18]

The functions of SMAS, as well as the classification and number of its types, are the subject of discussion in the scientific community. It is assumed that SMAS transmits facial contractions to the skin of the face and induces facial expression.[17] This function is consistent with the fact that SMAS is found only in humans and their closest evolutionary "relatives" - monkeys, since it is in these organisms that facial mimic activity is most pronounced among animals.[19]

Today, it is obvious that the extracted types of collagen correspond to the protein composition of type I collagen, consisting of α1, α2, β and γ chains [20] and can be used both locally and in the form of injections for inflamed tissues for the purpose of reconstruction. [21] Our data are consistent with the data of previous researchers. Indeed, collagen is present in the facial skin of animals, and the amount of this protein was different in different animals. However, collagen does not form a 3D structure characteristic of the human face. The study did not find any structures that even remotely resembled the SMAS. It is likely that the SMAS is an evolutionarily young, unique structure that is unique to the human face. Further research is needed to determine who first developed the SMAS and how it has changed over the course of evolution.

CONCLUSION

No structures similar to SMAS were found in the animals used in the experiment. Collagen of non-monkey animals on the face does not have the corresponding spatial organization, it is located laminarly. Most likely, this is due to the function performed by SMAS - the transmission of facial contractions to the skin. The structural organization of collagen on the human face is unique. Further research is required to accurately determine the evolutionary development of SMAS. The data obtained allow us to optimize the procedure for using collagen of animal origin as an additional source of nutrition for humans, as well as a structural intraoperative substrate

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Not Applicable

Competing interest
The authors declare that there are no competing interest.

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REVIEW ARTICLE

ANATOMICAL AND ANTHROPOLOGICAL VIEW OF TARGET POINTS AND LANDMARKS OF LOCAL ANESTHESIA IN THE MAXILLOFACIAL REGION:A NARRATIVE REVIEW

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ABSTRACT

Background: The anatomical-anthropological approach to local anesthesia target sites utilizes knowledge of individual anatomical variations in elderly patients to optimize conduction anesthesia efficacy while minimizing complication risks.

Aim: The aim of this study is to study the correlation of changes in anatomical and anthropological landmarks with the age of patients, finding constant points for successful anesthesia in dental practice.

Materials and Methods: The review is based on a retrospective analysis of articles on the anatomical and anthropological features of the structure of the maxillofacial region of the age group of patients, studied the location of the exit of the infraorbital, greater palatine, mental, and inferior alveolar nerves, taking into account the spatial relationships of bone structures and soft tissues in elderly patients. A systematic search was conducted in the PubMed, Wiley, Springer, eLibrary, Cyberleninka, Journals.lww.com, ResearchGate databases. The search period was from 1953 to 2024 for the following keywords: geriatric dentistry, age-related changes, infraorbital foramen, mandibular foramen, greater palatine foramen, mental foramen, trigeminal nerve, local anesthesia in Russian, English, German. More than 200 articles were reviewed, found 65 papers for the primary review, after a qualitative 38 publications were selected on age-related features inherent in patients of different age groups as the most relevant to the goals and objectives of the review. Review was conducted according to the PRISMA guidelines.

Results:A review of publications has revealed variability in anatomical-topographic parameters and the positioning of major foramina on the facial skull among elderly individuals across different geographic and chronological populations, as well as a close correlation between age-related changes in the maxillofacial region, tooth loss, and sexual dimorphism

Conclusion: To successfully achieve targeted anesthesia points and prevent complications during and after injection in elderly patients, it is essential to personalize the process of local conduction anesthesia and conduct preliminary radiographic and digital diagnostic methods.

Key words: age-related changes, infraorbital foramen, mandibular foramen, greater palatine foramen, mental foramen

INTRODUCTION

The increase in the life expectancy and quality of life of humanity has led to an increased trend of population aging. Thus, according to Russian statistic forecasts, by 2050 the number of people over 60 years of age will reach 50.0% of the total population of the country.[1] In the process of human aging, pathological and dystrophic changes develop in all organs and tissues, including the tissues of the maxillofacial region.[2] With age, certain areas of the facial skeleton are most prone to resorption processes, namely: the upper medial and lower lateral parts of the orbital margin, bone formations of the midface located in close proximity to the upper jaw and piriform aperture. On the lower jaw, the bigonial width and the width of the branches of the lower jaw remain unchanged, the height and length of the body of the lower jaw decrease with age, and the angles of the lower jaw increase [3]. A dentist needs knowledge of age-related and individual anatomical and topographic features of the maxillofacial region to correctly perform local anesthesia, since elderly patients are more susceptible to the risk of developing complications during and after the injection, and painless dental procedures have become an integral requirement for providing dental care to people of any age.[4,5] Individual anatomical variations in the shape, length and angle of the lower jaw show a correlation between the dose of local anesthetic for effective pain control during dental procedures.[6] It is worth noting that the topic under consideration was actively developed already in the 20th century and does not lose its relevance at present.

The aim of this study is to study the correlation of changes in anatomical and anthropological landmarks with the age of patients, finding constant points for successful anesthesia in dental practice.

MATERIAL AND METHODS

The review is based on a retrospective analysis of articles on the anatomical and anthropological features of the structure of the maxillofacial region of the age group of patients, studied the location of the exit of the infraorbital, greater palatine, mental, and inferior alveolar nerves, taking into account the spatial relationships of bone structures and soft tissues in elderly patients.

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More than 200 articles were reviewed, found 65 papers for the primary review, after a qualitative 38 publications were selected on age-related features inherent in patients of_different age groups as the most relevant to the goals and objectives of the review. The review was conducted according to the PRISMA guidelines (Figure 1).

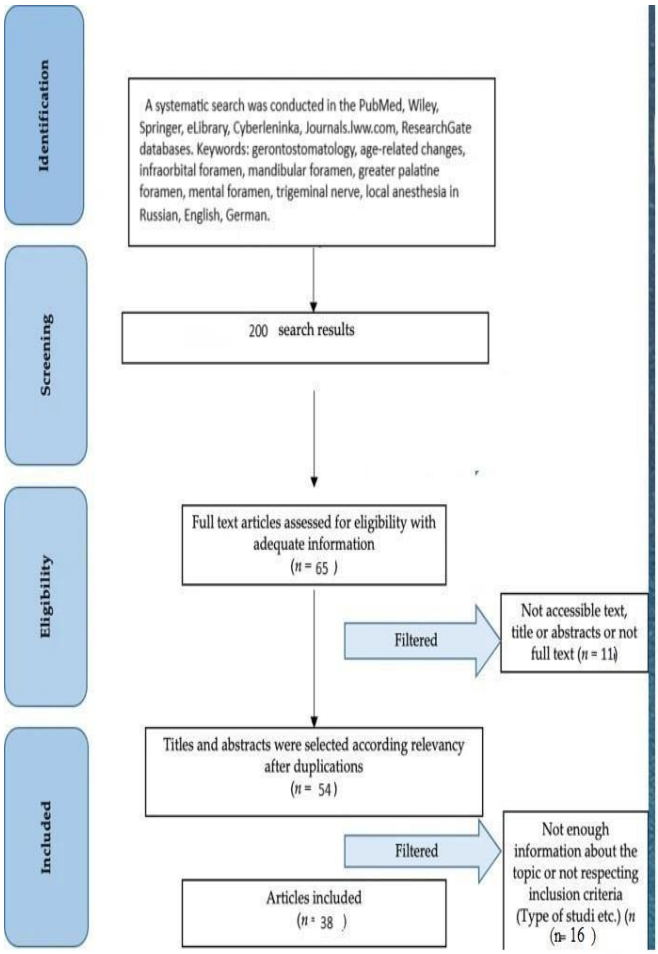


Figure 1. PRISMA flow chart

RESULTS

Analysis revealed significant geographical and temporal variations in craniofacial foramina positioning among elderly populations. Strong correlations emerged between maxillofacial aging patterns, tooth loss, and sexual dimorphism.

Studies conducted among individuals of Caucasian descent of different genders and ages have revealed that the cause of age-related changes in the position of ligaments, muscles, subcutaneous adipose tissue, and facial shape is the reduction of interbrow, orbital, maxillary, and piriform angles.[7] Research on the human facial skeleton from a medieval European osteological collection confirmed multiple age-related resorptive changes in the craniofacial region. The most active resorption occurs at the edges of the orbits

and piriform aperture, leading to an increase in their surface area and a decrease in the vertical height of the maxilla.[8]

Another study conducted on an Asian population corroborated these findings, although changes in the orbital and maxillary angles were less pronounced, while the piriform angle demonstrated more significant changes compared to European populations.[9]

The problem of atrophy in the maxillary alveolar process leads to functional impairments and disproportionality in the maxillofacial region, as well as shifts in the localization of anatomical landmarks: elements of the muscular system, neurovascular bundles passing through the palatal and incisive canals, and dental arch asymmetry. A reduction in the vertical dimension of the maxilla is accompanied by decreased fat tissue and collagen content in the skin, causing sagging of the soft tissues in the midface. After the age of 40–45, thinning of the bony walls of the maxillary sinuses is observed, eventually leading to increased pneumatization of the skull bones. Against the backdrop of age-related alveolar process atrophy, elderly individuals face an elevated risk of vascular damage and significant bleeding during dental procedures.[10]

Progressive bone loss, alveolar bone atrophy and osteoporosis, facial structural changes, altered appearance, and issues with chewing and speech are observed in elderly edentulous patients. Alveolar bone atrophy is more extensive and progresses faster in the mandible than in the maxilla.[11] A correlation has been established between secondary edentulism in the elderly and reduced vertical parameters of the mandible, mandibular ramus height, and an increased gonial angle. The mandibular angle increases with age—from 97 to 135 degrees—due to resorption of the lower border of the angle near the junction of the body and ramus. It has been found that with age, the chin becomes more prominent and shorter in both men and women. The reduction in vertical dimensions of the mandibular ramus is associated with an increased distance between the mandibular angle and the hyoid bone, likely due to condylar process resorption or osteoarthritis.[12]

In a cross-sectional group analysis of 160 skulls, Bartlett et al. observed that facial width, depth, and height either increase or remain unchanged in the elderly. These findings support the theory that the skull continues to grow, albeit at varying rates from infancy to old age. Changes in craniofacial morphology closely correlate with tooth loss and are sex-dependent. In women, nearly all transverse cranial dimensions increase with age, whereas no such changes occur in men.[13] A study by Daniela Garib et al. provided further evidence that the craniofacial complex continues to change from early to mature adulthood during aging, acquiring anatomical and morphological differences between men and women.[14]

Facial muscles in young individuals exhibit broad, convex contours, which flatten with age due to restructuring of the subcutaneous adipose tissue in the Bichat's fat pad region. Additionally, in cases of partial or complete secondary edentulism—common in the elderly—the function of masticatory muscles, particularly the masseter and temporalis, declines.[15] Hypofunction of the masticatory muscles and subsequent changes in muscular engrams lead to distal displacement of the mandible and loss of lower facial height.[16] All patients experience age-related changes characterized by reduced overall muscle mass.[17] With aging, skeletal muscles atrophy by an average of 50%, unlike facial muscles, which maintain constant tension, undergo elongation, increased tone, and reduced amplitude.[18]

Changes in the soft tissue profile of the face occur between ages 5 and 45, with earlier changes observed in females (10–15 years on average) compared to males (15–25 years).[19] According to Pecora NG, Baccetti T, and McNamara JA Jr., structural changes in soft tissues—including drooping of the nasal tip and columella, elongation, and thinning of the upper lip—are defining features of craniofacial changes across age groups.[20] Analysis of 88 human facial scans (ages 26–90) by Windhager S, Mitteroecker P, Rupić I, Lauc T, Polašek O, and Schaefer K concluded that facial aging progresses faster in women than in men.[21]

Literature indicates that age-related musculoskeletal changes in the maxillofacial region determine the positioning of major foramina in the elderly. A retrospective analysis of morphometric characteristics of the infraorbital foramen and cone-beam computed tomography data from 125 patients (ages 18–75) by S Dağıstan, Ö Miloğlu, O Altun, and EK Umar revealed that a round infraorbital foramen shape was common in the 30–39 age group, while an oval shape was more frequent in those aged 20–29, with both shapes more prevalent in females.[22] The study also found the infraorbital foramen diameter to be largest in the 40–49 age group.

Data on the topography and asymmetry of the infraorbital foramen from A. Gawlikowska-Sroka et al. confirm geographical and chronological variations

among European populations, which may aid in targeting injection points for regional anesthesia.[23] However, the classical infraorbital anesthesia technique lacks precision in aligning needle insertion with the target site, as it disregards individual variations in vascular and neural anatomy. Variability in the anatomical-topographic parameters of the infraorbital foramen and facial skeletal structure necessitates personalized approaches to regional anesthesia, incorporating radiographic and digital diagnostic methods.[24,25]

Morphometric studies of the greater palatine foramen show that the most common shapes among both sexes are oval (elongated anteroposteriorly), teardrop, and round. Less frequent shapes include crescent, triangular, rhomboid, and helical. The diameters of the greater palatine foramen and the length of its canal are larger in men, demonstrating significant sexual dimorphism.[26]

Variability in the location and shape of the greater palatine canal—including undulating, funnel-shaped with inferior curvature, hourglass, zigzag, crescent, and straight—is independent of age and sex.[27]

Anatomical-topographic features of the mandibular foramen, and thus the injection site for regional anesthesia, exhibit age-related differences. In 3-year-olds, the mandibular foramen is located 4.12 mm below the occlusal surface of molars, ascending with age to reach the occlusal level by age 9. In adults, it lies 4.16 mm above the occlusal surface. At age 3, the foramen is positioned at one-third the ramus height, reaching the midpoint in adulthood, with size varying from 67.8% at age 3 to 61.7% in adults.[28?29] In individuals with reduced anterior facial height, the mandibular foramen may be even higher, located at 60% of the ramus width from its anterior edge. The vertical distance between the mandibular foramen and occlusal plane shows significant variability, particularly with aging.[30]

Sandhya S.T. and Karpagam identified asymmetry in the position of the mandibular foramen relative to landmarks on the left and right rami, ranging from 13% to 20%, irrespective of age.[31] According to Ditti J. Mary and Karthik Ganesh Mohanraj, a correlation exists between the gonial angle and mandibular foramen position, offering valuable insights for successful inferior alveolar nerve block and dental procedures.[32] Other studies have revealed anatomical variability in the macrostructure and topography of the inferior alveolar nerve in adults and the elderly, manifested by high (4.0–11.0 mm) and low (22.0–33.0 mm) origins relative to the oval foramen, as well as variations in branching (single or dual trunks).

The number of branches to the teeth, interalveolar septa, and gums from the inferior alveolar nerve also decreases significantly with age and tooth loss.[33]

The mental foramen, a bilateral opening on the anterior mandible, transmits the terminal branch of the inferior alveolar nerve (mental nerve).[34,35,36] Its position relative to the mandibular borders remains relatively constant, aiding in nerve localization even in elderly patients. Achilandeshwari and Ranganath noted that the mental foramen is farther from anatomical landmarks in dentate jaws than in edentulous ones.[37] Mental foramen topography varies significantly across ethnic and age groups depending on sexual dimorphism and vertical facial dimensions (short, normal, or long face), which holds clinical relevance in dentistry.[38]

CONCLUSION

To successfully achieve targeted anesthesia points and prevent complications during and after injection in elderly patients, it is essential to personalize the process of local conduction anesthesia and conduct preliminary radiographic and digital diagnostic methods.

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Conflict of interest

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ORIGINAL ARTICLE

MEDICAL ASSESSMENT IN THE FIELD OF SOCIAL SECURITY AND CARE OF EDENTULOUS PEOPLE

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Oral diseases are among the most frequent pathologies which can impact health and economic implications, particularly reducing quality of life. The most prevalent oral diseases are dental caries, periodontal disease, tooth loss, and oral cancer.
Prevention represents the only mean to avoid or reduce more serious problems.
Unfortunately, dental care is not provided early and this has implications both on the oral health and on the economy, not only for the patient but also for the social security system.
The aim of this study was to analyze which oral pathologies most influence social security.

Keywords: dental caries, oral carcinoma, social security implications.

INTRODUCTION

Oral diseases are among the most widespread diseases that can affect the quality of life of those affected and can lead to serious health and economic burdens. The most common oral diseases are dental caries, periodontal disease, tooth loss and tumors of the oral cavity[1].
The patients most affected by oral diseases are children living in poverty, socially marginalized groups and the elderly and all those who, for various reasons, have little access to dental care. The consequences of untreated chronic oral diseases are often serious and can include pain, infections, difficulty chewing and therefore difficulty eating with reduced quality of life, missed school days, interruption of family life and decreased work productivity in adults. Although oral diseases are largely preventable, this is still not the case today. In many low and middle-income countries, oral diseases remain largely untreated because treatment costs exceed available resources[2].
Oral diseases are undoubtedly a public health problem, with particular concern about their increasing prevalence in many low and middle-income countries linked to wider social, economic and commercial changes; however, unfortunately, dental care is often not as accessible in many populations with many neglecting treatments. Depending on age, some pathologies are more frequent than others, therefore prevention should be aimed at different age groups[3].
The aim of this study was to analyze which pathologies were more frequent based on age and their medico-legal implications.

Children

In children, the most common dental pathology is dental caries. Dental caries is a destructive process of the hard tissues of the tooth and its etiology is multifactorial[4,23].

A late diagnosis of caries may cause deterioration of general health of children and an inevitable reduction in the quality of life. Caries may cause pain, disturbed sleep and/or infections with abscesses. It is often necessary to perform extractions of deciduous teeth and early loss can cause malocclusion in children. Furthermore, all these conditions can compromise nutrition leading to weight loss, impaired speech, loss of learning and of days at school with, indirectly, repercussions on the parents. Another important negative aspect is "inefficient" chewing; furthermore, unchewed foods are swallowed almost whole, with exposition of children to higher risk of suffocation [5,7].

Adults

Adults, in addition to caries, are affected by periodontal diseases, precancerous lesions and tumors. If untreated, periodontal disease may lead to tooth loss resulting in difficulty with chewing, speaking and pain in the temporomandibular joint[4,8]. Regarding tumors of oral cavity, they are benign or malignant.

The benign tumors have a slow, gradual and disruptive growth; conservative treatment is not always the solution specially in case of relapse: for this reason it is better to perform a mandibular or maxillary surgical resection.

The malignant tumors of oral cavity can have epithelial or mesenchymal origin; they always need a surgical resection, often with chemotherapy and radiation treatment. Even if radiation treatment has an important positive effect on disease, it has a negative effect on bone metabolism and adjacent tissues, so that is difficult to place dental implants[22].

Elderly people

As regards to the elderly, they are those with higher risk of chewing problem and its impact on nutrition. Taste and smell are fundamental elements for dietary selection, especially for adults and elderly people whose appetite is reduced for aging. In older adults with reduced appetite, possibly due to disease, taste is one of the most important factors in food preference, selection and consumption [5]. This attitude within the elderly population can lead to dietary restrictions that could have a negative impact on nutritional and health status [6]. The decrease in the quantity and quality of ingested foods and the inability to detect harmful tastes and odors make elderly patients vulnerable to foodborne illness, loss in somatosensory perception and oral sensitivity, which is deleterious to their ability to discriminate and differentiate the textures of foods with the risk of choking [7,8,9]. Some medications are known to cause loss of taste acuity (hypogeusia), distortion of taste (dysgeusia) or loss of sense of taste (ageusia). Unfortunately, smell and taste disorders in the elderly are commonly overlooked, as they are not considered life-critical[10,11,12]. Decreased olfactory function is a contributing factor to increased age in accidental gas poisonings and explosions that can endanger public safety[13,14,15]. Decreased smell and taste result in appetite suppression resulting in weight loss, malnutrition, reduced immunity and deterioration in medical conditions[16,17]. It has been reported that elderly people require from two to three times higher concentration of salt. In older adult's diet, higher salt and sugar intake can aggravate dangerous health conditions[18,19,20,21].

A proposal for medical assessment in the field of social security and care of partial or total edentulous subjects

The main aim of this study was to provide a proposal for medical assessment in the field of social security and care of partial or total edentulous subjects in complex clinical disease pictures [24,25,26],

Edentulism is a condition characterized by a partial or a total loss of teeth.

Total edentulism can be firstly related to aging but even to different pathologies, first of all periodontal disease for which we have people over 40 years old with loss of teeth in 35 percent of cases.

Total edentulism can also be due to serious systemic disorders, such as tumors, HIV, lymphomas, leukemias, neutropenias, malnutrition and denutrition, all bad conditions of health.

Partial edentulism can be due to genetic factors or assumption of elements that causes a loss of teeth.

Nowadays, the prevalence and the incidence of edentulism and the loss of teeth in European countries is unknown.

In Italy, the prevalence of edentulism is about 10,9%, higher in women (12,5%) than in men (9,2%).

The loss of teeth increases with age, significantly after age 60.

As a matter of fact, we don't have the loss of dental elements until age 44; it is about 3% from age 45 to 54, 10,5% from age 55 to 64 and 22,6% from age 65 to 69 [19,23].

The causes of edentulism, partial and total, can be distinguished in:

- post-traumatic;
- neoplastic;
- concerning bone metabolism;
- inflammatory.

For a medical assessment in the field of social security and care of subjects affected by edentulism, the functional impact of edentulism in relation to chewing, phonetics and breathing must be underlined.
The Italian Ministerial Decree 5 February 1992 denotes a condition of partial or total edentulism, further divided into aidable and unaidable.
The Law 12 June 1984 number 222 provides following benefits:

- ordinary allowance of invalidity, for people with residual working capacity (semi-specific or attitude skill) less than 1/3 of the total;
- ordinary inability pension, for workers no longer able to carry out any work;
- monthly allowance for personal and continuous assistance for those receiving ordinary inability pension who are unable to walk because of their disability without the permanent help of an accompanying person or because they are unable to carry out their daily life activities, but who need continuous assistance.

Pursuant to article 3, paragraph 3 of Law 5 february 1992 number 104 a person with a disability is someone who has long-lasting physical, mental, intellectual or sensorial impairments which, in interaction with barriers of various kinds, can hinder full and effective participation in different life contexts on an equal basis with others, ascertained following the basic assessment.

It must be highlighted the case of a person of working age who, following multiple trauma with involvement of facial massif, suffers the traumatic or therapeutic avulsion of all dental elements resulting in severe masticatory disorders up to the ultimate clinical condition of malnutrition and cachexia associated with malabsorption with inability to carry out continuous and profitable work activity, partially or totally, such as to satisfy the conditions for eligibility for economic benefits in the field of social security.

In the area of care, there is the example of a citizen suffering from malignant neoplasm with severe prognosis, already undergoing radiation therapy, with consequent edentulous condition, who can be recognized civilian invalid.

Further examples of care provision can be substantiated in the hypothesis of a citizen suffering from Alzheimer's disease and edentulous such as not to allow a diet useful to their livelihood, hence the proposal to recognize the accompanying allowance[10].

The accompanying allowance can be recognized when the medical examiner determines an edentulous person suffering from psychosis without the ability to self-determination in useful daily sustenance.

The above example in the context of the recognition of the condition of social disadvantage is to be proposed as certainly attributable to a condition of gravity as corresponding to the conditions of article 3, paragraph 3 of Law 5 february 1992 number 104 "if the handicap, single or multiple, has reduced the personal autonomy, correlated to the age, so as to render necessary a permanent, continuous and total assistance intervention in the individual field or in that of relation" of the person.

The workers of the show that cannot be treated, because they suffer from pathologies that contraindicate the implant therapy or because they suffer from health problems that prevent their adaptation to the prosthesis, are forced to edentulous: the latter, although alone does not give the right to the economic benefit, must nevertheless be assessed both in the dysfunction that it causes and in the complications.

These workers encounter digestive and phonetics problems frequently developing a feeling of insecurity and difficulty in relating with consequent restriction of their social and work role (in particular, actors, radio and television hosts, wind instrument players and others in the entertainment world who need not only aesthetic integrity, but also of the phonetic one that in case of dental pathology and/or traumas with consequent dental fracture, can be compromised).

In a judgment of the Court of Trapani there is the recognition of the monthly assistance allowance from the date of submission of the administrative application in a man aged 63, suffering from ischemic heart disease (hypertensive), already subjected to coronary angioplasty with angina stress, diabetes mellitus not insulin – dependent, obesity of II class, with polyarthropathy, Parkinson's disease with slight slowdown ideo-motor, in association with partial unaidable edentulism (TRAPANI COURT, Judgment number 495/2022 of 17.11.2022)".

DISCUSSION

"The mouth is connected to the rest of the body" is a phrase often used as an important reminder of the systemic connection between an individual's oral health

and overall health. In reality this is true considering that oral cavity problems can lead to serious health implications. From a social security point of view, it is necessary to evaluate the functional impact of a condition of edentulism (partial or total) on the complex clinical picture to which the subject is affected, in order to recognize the economic benefits provided by law. This would have a lower incidence on the disease with a lower increase in spending money.

Although the oral health of children in Italy has improved in recent years, tooth decay remains the most widespread chronic childhood pathology, five times more common than asthma and is prevalent among children belonging to less well-off families. Despite scientific evidence demonstrating that a state of good oral health protects both general health and quality of life during pregnancy, currently in Italy women do not perform dental care as part of their prenatal preparation. On the contrary, in the United States the American Dental Association and the American College of Obstetricians and Gynecology prescribe a specific dental care protocol for pregnant women [27].

No similar protocol is yet carried out in Italy, where the role of oral health in the general health of the woman and her unborn child tends to be underestimated. The understanding of maternal and fetal physiology indicates that providing adequate dental care during pregnancy does not represent a risk, but rather that not providing can represent an increase in risk for the mother and the future unborn child. The American Academy of Periodontology, for example, prescribes the prevention of oral pathologies already in the initial phase of pregnancy in order to prevent the onset of acute infections or sources of sepsis. The timing of such care is critical, as the oral health of pregnant women impacts the health of the fetus. Prevention could represent the real solution to avoid serious dental problems and the various implications [27,28,29].

Table 1. Summary of the most common oral diseases that impact on social security

ORAL DISEASES	SOCIAL SECURITY IMPLICATION	SYMPTOMS	TREATMENT
Dental caries	Missed days at school, loss of parents days at work	Pain, infections	Pharmacological therapy, dental care
Dental caries, periodontal disease, oral cancer	Job loss	Pain infection	Dental care, prevention
Loss of teeth	Hospitalization	Hypogeusia/ dysgeusia/ ageusia Risk of choking	Restore the occlusion

CONCLUSION

In light of the considerations made, prevention is the most concrete expression of a far-sighted treatment system, which foresees and provides for the future development of the child, who, going through multiple stages of development, will become an adult. If the prevention program is implemented from the first months of life, it may avoid factors that are potentially harmful to the child's development; this will result in a lower incidence of future problems relating to the subject's health and a lower economic commitment for families and the system healthcare.

For a medical assessment in the field of social security and care of subjects affected by edentulism, the functional impact of edentulism in relation to chewing, phonetics and breathing must be highlighted.

DECLARATIONS

Ethical approval and consent to participate – The study was approved by the Institutional Ethics Committee.

Competing interest

The authors declare that there are no competing interest.

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REVIEW ARTICLE

THE EFFICACY OF LASER THERAPY IN LABIAL FRENULECTOMY COMPARED WITH CONVETIONAL SURGERY IN CHILDREN AND ADOLESCENTS: A SYSTEMATIC REVIEW

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Background: Labial frenulectomy is one of the frequently utilized procedures to treat complications from a tight or projecting labial frenulum that can hinder speech, oral hygiene, or orthodontic care. The surgery has traditionally used a scalpel. But laser therapy has emerged in popularity because of its minimally invasive character and enhanced patient comfort. This review discusses the comparison between laser therapy and the traditional method of surgery with regard to clinical outcome and patient satisfaction.
Methods: A thorough search was conducted using three major databases PubMed, Scopus, and Web of Science to identify studies that compared laser-assisted and traditional surgical approaches for labial frenulectomy. The review focused on differences in pain levels, bleeding, healing duration, procedure time, and post-surgical complications.
Results: Out of a total of 226 studies found in an initial search, 7 were included on the basis of predetermined criteria. The majority of studies included in the reviews stated that laser therapy led to less bleeding, rapid healing, less postoperative discomfort and shorter surgery times. Variability in study design, laser type and the means of evaluating outcomes rendered comparisons difficult.
Conclusion: Laser therapy would seem to have a number of advantages over conventional methods of surgery in terms of patient comfort and recovery time. Despite this, current evidence is weak. Stronger, well-conducted studies are required to fully evaluate long-term efficacy and to formalize standardized best practice.

Keywords: labial frenulectomy, laser surgery, conventional surgery, systematic review, oral surgery, wound healing.

INTRODUCTION

The labial frenulum, so frequently a small, forgotten anatomical feature, may exert a surprisingly decisive effect upon both function and aesthetics in the mouth. Bounded between the inside of the upper lip and the middle incisors' gingiva, this tissue fold can, when abnormally attached, obstruct speech, feeding, hygiene, stability in the area after orthodontics, as well as facial harmony.[1-6] High or thick labial frenal attachments in children or adolescents who are having orthodontic treatment can be more than clinically consequential, but can interfere with self-esteem, habits, and everyday living. A frenectomy, in these instances, is usually the surgery advised, but one intended not so much to treat, but rather to relieve the limitation in function.[7-9]

Historically, a traditional scalpel technique has been used for frenectomy. This technique, while more traditional in use, has some disadvantages. Scalpel surgery is linked with higher intraoperative bleeding, sutures required, healing time, and discomfort in the postoperative period. In pediatric cases in particular, the apprehension of undergoing such procedures is rather high, further bringing into relief the necessity of methods that are pain-reducing, traumatic, and timesaving during recovery.[10-12]

Over the last decades, laser technology has presented a promising alternative to the use of traditional methods involving a scalpel. CO_2 lasers[13], diode lasers[14], Er:YAG, and Nd:YAG lasers[15] are among the most frequently used lasers in frenectomy procedures, each possessing distinct properties and attributes. Several studies have identified laser-assisted frenectomy as resulting in less bleeding, shorter surgery time, less suture requirements, less postoperative pain, and faster healing.[16,17] This has inspired increasing enthusiasm among clinicians for the use of laser therapy in regular practice for soft tissue oral surgery.

One of the most immediate, if not the earliest, advantages of laser surgery in the use of soft tissue is the precision of cutting with the immediate cauterization of blood vessels, decreasing bleeding a great deal, as well as improving visualization for the clinician. In labial frenectomy, this is especially valuable in light of the vascular nature of the labial frenum, as well as the problem of operating within a small area, in the younger child. A randomized controlled trial by Xie et al. made a comparison between laser (dual wavelengths, Er:YAG, Nd:YAG) use and traditional use of a scalpel in 34 children. In their study, they reported reduced time of surgery, together with decreased intraoperative and postoperative discomfort in the laser treatment. Patients who received laser frenectomy experienced less discomfort during and after the surgery, as well as improved chewing, speaking, and scar formation at follow-up.[18]

In a randomized clinical trial conducted by Sarmadi et al., the efficacy of the Er:YAG laser was compared to that of the conventional scalpel technique in patients aged 7 to 19 years. The outcomes were consistent with prior evidence, demonstrating that laser-assisted frenectomy resulted in significantly reduced intraoperative bleeding and shorter procedural duration. Notably, although the immediate postoperative wound area appeared larger in the laser group, this difference resolved rapidly, with no discernible discrepancy between groups by postoperative day five. Both groups reported high levels of patient satisfaction; however, the laser technique was particularly favored due to its procedural efficiency and minimally invasive nature.[19]

Yadav et al. provided additional insights by comparing the Nd:YAG laser technique with the conventional scalpel method in adult patients. Their findings were consistent with outcomes reported in pediatric populations, demonstrating that laser-assisted procedures were associated with reduced intraoperative bleeding, lower postoperative pain scores, decreased need for analgesics, and accelerated wound healing. A particularly noteworthy aspect of their study was the emphasis on patient-centered outcomes; individuals treated with the Nd:YAG laser frequently did not require sutures or periodontal dressings, highlighting an important advantage in terms of postoperative comfort and clinical efficiency.[20]

Extending the scope of investigation, Ginini et al. explored the parental perspective in a cohort study involving preschool-aged children undergoing frenectomy performed with either a CO_2 laser or a traditional scalpel. Utilizing a mobile application to systematically record pain levels, parental satisfaction, and perceived clinical improvement over a one-month follow-up period, the study revealed that although children in the scalpel group reported lower immediate postoperative pain scores, the laser group was associated with significantly higher levels of parental satisfaction and perceived long-term improvement. These findings suggest that despite potentially greater initial discomfort, laser-assisted frenectomy may offer superior long-term outcomes and reduced complication rates from the caregivers' standpoint.[21] Sobouti et al. conducted a comprehensive investigation into the impact of laser wavelength on

clinical outcomes, comparing two diode lasers 445 nm (blue light) and 980 nm (infrared) with the conventional scalpel technique. In a robust study involving 174 patients, both laser modalities demonstrated superior results relative to the scalpel group in terms of intraoperative bleeding, patient discomfort, postoperative pain, and healing progression. Notably, the 445 nm diode laser outperformed the 980 nm variant, underscoring the critical role of wavelength selection in optimizing laser-assisted surgical outcomes. This type of intra-technology comparison is essential for clinicians aiming to refine their procedural techniques and make informed decisions regarding equipment acquisition.[22]

The collective analysis of existing literature clearly demonstrates that laser therapy, in its various modalities, provides a range of tangible advantages over conventional scalpel-based frenectomy. These benefits include reduced intraoperative bleeding, shorter procedure duration, accelerated postoperative healing, and decreased need for sutures. For pediatric patients, these outcomes translate into diminished procedural anxiety and fear, whereas adult populations benefit from reduced postoperative discomfort and minimal downtime. From the clinician's standpoint, improved surgical visibility, enhanced patient satisfaction, and greater procedural efficiency are notable strengths.[23]

Nevertheless, despite the growing body of positive evidence, several methodological inconsistencies across studies limit the generalizability of conclusions. Heterogeneity in study designs, patient demographics, and outcome measures poses significant challenges. Some investigations rely heavily on subjective metrics such as Visual Analog Scale (VAS) scores for pain, while others emphasize objective parameters like blood loss volume and operative time. Moreover, the diversity in laser types, wavelengths, and surgical protocols complicates direct comparisons. Critically, long-term outcomes such as relapse rates or post-frenectomy orthodontic stability, remain insufficiently explored in the current literature. It is also essential to acknowledge the limitations inherent to laser therapy. High equipment costs, the necessity for specialized training, and safety considerations particularly in pediatric settings must be carefully weighed prior to widespread clinical implementation. Not all dental or surgical practices may possess the infrastructure or expertise required to adopt this technology, and the associated learning curve can be substantial depending on the specific system utilized.[24]

In light of these considerations, the objective of this systematic review is to synthesize current clinical evidence comparing laser-assisted techniques with conventional scalpel-based approaches specifically in the context of labial frenectomy. By analyzing a diverse range of randomized controlled trials and comparative studies across different age groups, geographic regions, and clinical environments, this review aims to determine whether the advantages of laser-assisted procedures are consistent and significant enough to inform future clinical guidelines. Furthermore, by incorporating both clinical outcomes and patient-reported measures such as pain perception and satisfaction, we seek to provide a comprehensive perspective to aid informed decision-making for both clinicians and patients.

Ultimately, while no single technique may be universally optimal, the expanding body of evidence favoring laser-assisted frenectomy supports its consideration as a preferred modality particularly in pediatric dentistry, orthodontic management, and contexts emphasizing patient-centered care. This review aims to consolidate existing data, identify current research gaps, and offer clearer direction for evidence-based clinical practice in the evolving field of soft tissue oral surgery.
The aim of this study was to evaluate the efficacy of laser therapy in labial frenectomy in comparison with conventional surgical methods.

MATERIAL AND METHODS

Eligibility criteria

The studies were selected with the guidance of the PICO framework (Population, Intervention, Comparison, and Outcome).

Population (P): Human studies were taken into account.

Intervention (I): The intervention in question was laser treatment for labial frenectomy.

Comparison (C): The control was traditional surgery methods, e.g., procedures involving a scalpel.

Outcome (O): The main outcome measured was the clinical effect of laser treatment versus traditional surgery for labial frenectomy.

Only studies with documentation of clinical outcomes after labial frenectomy completion were considered. The exclusion criteria were:

(1) patient studies in cases of systemic diseases with the potential to affect healing (for instance, chronic inflammatory or autoimmune disorders);

(2) studies in patients undergoing any other concurrent oral surgery procedures;

(3) lingual frenectomy or other oral frenectomies studies;

(4) individuals with a history of craniofacial syndrome or abnormalities;

(5) studies with removable prosthetic appliances (for example, wearers of a denture);

(6) cross-over study design;

(7) published in a language other than English;

(8) articles with no full text available (e.g., conference proceedings, posters);

(9) animal or in vitro model studies;

(10) reviews, such as systematic reviews, or narrative reviews;

(11) case reports or case series.

Search strategy

A detailed search of three digital databases, Web of Science, PubMed, and Scopus, from inception through April 2025 was conducted utilizing the search strategy shown in Table 1. Furthermore, a hand search of the reference lists of studies meeting the selection criteria as well as pertinent systematic reviews was also conducted to find any further candidate articles. The systematic review was performed adhering to the methodological criteria described in the Cochrane Handbook for Systematic Reviews of Interventions and was reported in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020. The review protocol was registered prospectively in the International Prospective Register of Systematic Reviews (PROSPERO), with the number CRD420251025588.

Table 1. Search strategy

PubMed
Search: (Labial Frenulum) AND (laser) ("labial frenum"[MeSH Terms] OR ("labial"[All Fields] AND "frenum"[All Fields]) OR "labial frenum"[All Fields] OR ("labial"[All Fields] AND "frenulum"[All Fields]) OR "labial frenulum"[All Fields]) AND ("laser s"[All Fields] OR "lasers"[MeSH Terms] OR "lasers"[All Fields] OR "laser"[All Fields] OR "lasered"[All Fields] OR "lasering"[All Fields])
Scopus
ALL (frenulectomy AND laser)
Web of Science
(ALL=(frenulum surgery)) AND ALL=(laser)

Data extraction

Data extraction from the studies was performed by two independent reviewers (MPS and GM) in a Microsoft Excel spreadsheet specifically tailored for this review. Disagreement between the two reviewers was settled by consulting a third reviewer (MMM) to achieve consensus. The following data were extracted: 1) First author; 2) Year of publication; 3) Nationality; 4) Number of study participants; 5) Treatment tool; 6) Clinical relevance; 7) Age (Table 2).

Quality assessment

In this review, the risk of bias was assessed using the RoB 2 tool (Revised Cochrane Risk of Bias Tool for Randomized Trials), as all included studies were randomized controlled trials (RCTs). This validated tool evaluates five domains of potential bias: (1) bias arising from the randomization process, (2) bias due to deviations from intended interventions, (3) bias due to missing outcome data, (4) bias in measurement of the outcome, and (5) bias in selection of the reported result. The use of a standardized tool ensured consistent and rigorous evaluation of methodological quality across all included RCTs.

All studies were independently reviewed by two trained reviewers (MPS and GM). In cases of disagreement, a consensus was reached through discussion. If disagreement persisted, a third reviewer (MMM) provided the final decision. This systematic and standardized approach ensured a transparent and reliable evaluation of methodological quality, strengthening the validity of the review findings.

RESULTS

Study characteristics

226 studies were identified at the end of the research. As illustrated by the PRISMA 2020 flowchart in Figure 1, we chose only 7 studies to draw up the present systematic survey. We excluded 107 articles before the screening: 52 were reviews, 34 were case reports and editorials, and 12 were not in English.The remaining articles (n= 119) were selected for the title and abstract screening to evaluate whether they meet the PICO criteria.16 articles were excluded as duplicates and 99 records were assessed for eligibility. Among these, 92 were excluded: 36 did not respond to PECO, and 56 were off topic. The included studies have been published between 2019 and 2023. A total of 7 studies were included in the review. All studies compared the efficacy of laser therapy in labial frenulectomy compared with conventional surgery. Table 2 summarizes the main characteristics of all the studies included in the present systematic review (as reported in the paragraph data extraction) (Figure 1).

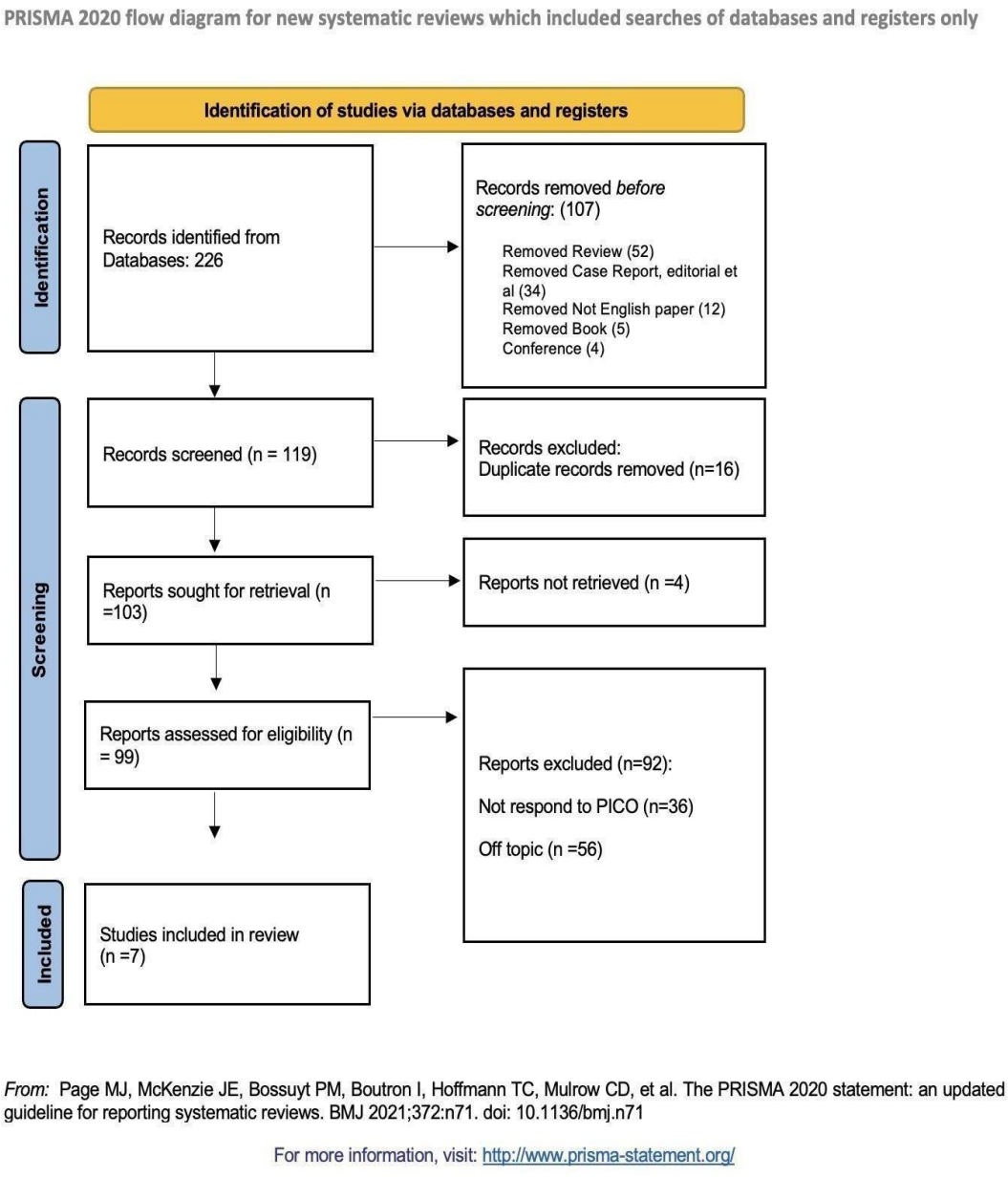


Figure 1. PRISMA 2020 Flow Diagram of Study Selection

Table 2. Data extraction

First author	Year of publication	Nationality	Number of study participants	Treatment tool		Clinical relevance	Age
Xie L	2022	China	34	Er:YAG and Nd:YAG laser vs scalpel		Laser reduced surgical time, pain, and improved healing	5-10
Sarmadi R	2021	Sweden	40	Er:YAG laser vs scalpel		Laser caused less bleeding and similar satisfaction	7-19
Ginini JG	2023	Israel	49	CO2 laser vs scalpel		Laser gave higher satisfaction despite more initial pain	2-6
Yadav RK	2019	India	20	Nd:YAG laser vs scalpel		Laser showed less pain and bleeding, comparable healing	
Sobouti F	2024	Iran	174	445 nm & 980 nm diode laser vs scalpel		445 nm diode laser had best healing and least pain	Average of 18.6
Calisir M	2018	Turkey	40	Nd:YAG laser vs scalpel		Laser provided lower pain and better patient comfort	Average of 22.6
Kara C	2008	Turkey	40	Nd:YAG laser vs scalpel		Compared patient perceptions between techniques	Average of 16.45

Main findings

This systematic review assesses the comparative efficacy of laser-assisted labial frenulectomy versus conventional scalpel surgery, based on data extracted from seven clinical studies. Xie et al. (2022) conducted a randomized clinical trial involving 34 pediatric patients (aged 5–10 years), comparing dual wavelength Er:YAG and Nd:YAG laser techniques to the conventional scalpel method. Laser-assisted frenectomy significantly reduced surgical duration (mean: 224 ± 59 seconds) compared to scalpel surgery (mean: 740 ± 168 seconds). Visual Analog Scale (VAS) pain scores were significantly lower in the laser group during surgery

and on the first postoperative day ($p < 0.05$). By postoperative day seven, no significant differences were observed in pain during chewing or speaking. Healing was complete in all cases, except for one scar formation in the scalpel group.[18]

Sarmadi et al. (2021) performed a randomized, single-blind clinical trial with 40 participants, comparing Er:YAG laser and scalpel techniques. The laser group exhibited significantly reduced intraoperative bleeding (mean gauze weight: 0.16 g vs. 0.44 g, $p < 0.05$) and shorter operative time (mean: 6.7 minutes vs. 10.5 minutes, $p < 0.01$).

Although the laser group presented with a larger wound area immediately postoperatively, no differences in healing were noted by day five. Patient satisfaction was high and comparable between groups.[19]

Ginini et al. (2023) evaluated 49 children (aged 2–6 years) undergoing either labial or lingual frenectomy with CO_2 laser or scalpel. While pain levels during the first 72 hours were lower in the scalpel group (mean VAS: 3.6 vs. 4.9), parental satisfaction and perceived improvement at one month were significantly higher in the laser group (mean satisfaction score: 9.1 vs. 7.2, $p < 0.001$).[21]

Yadav et al. (2019) studied 20 adult patients, reporting that Nd:YAG laser frenectomy was associated with significantly reduced intraoperative bleeding ($p = 0.016$), lower pain scores ($p = 0.016$), and a decreased need for analgesics ($p = 0.008$). However, no statistically significant difference in healing was observed at the three-month follow-up ($p = 0.095$).[20]

Sobouti et al. (2024) conducted a large-scale randomized trial with 174 participants, comparing 445 nm and 980 nm diode lasers against scalpel surgery. The 445 nm laser group achieved superior outcomes, including significantly lower pain scores both immediately and on day seven ($p < 0.05$), as well as enhanced tissue healing at days 7 and 30. Both laser groups demonstrated significantly reduced intraoperative bleeding and functional discomfort compared to the scalpel group (all $p < 0.05$).[22]

Calisir and Ege (2018) applied a split-mouth design in 40 patients. Sites treated with Nd:YAG laser reported lower VAS pain scores on the day of surgery and postoperative day one (mean VAS: 2.1 vs. 4.5, $p < 0.05$), along with improved comfort during mastication and speech.[25]

Kara (2008), in a comparative study, observed that Nd:YAG laser-treated patients reported greater postoperative comfort and satisfaction. Although precise VAS scores were not provided, the study emphasized the advantages of improved hemostasis and reduced need for sutures with laser surgery.[26]

Collectively, these findings reinforce the clinical efficacy of laser-assisted frenectomy in reducing surgical duration, intraoperative bleeding, and postoperative pain, while simultaneously enhancing patient satisfaction, particularly among pediatric populations.[27]

Quality assessment and risk of bias

Among the included studies, all seven were randomized controlled trials and were evaluated using the RoB 2 (Risk of Bias 2.0) tool. These studies included those conducted by Xie et al. (2022), Sarmadi et al. (2021), Ginini et al. (2023), Yadav et al. (2019), Sobouti et al. (2024), Calisir & Ege (2012), and Kara C. (2008).

Each study was independently reviewed by two assessors, with final judgments made through consensus or, when necessary, adjudication by a third reviewer. As illustrated in Figures 2 and 3, the majority of domains were judged at low risk of bias, with only isolated concerns identified in the randomization and outcome-measurement domains of a few trials. Employing this standardized, validated tool ensured a transparent and reproducible assessment of methodological rigor across all randomized designs (Fig 2,3).

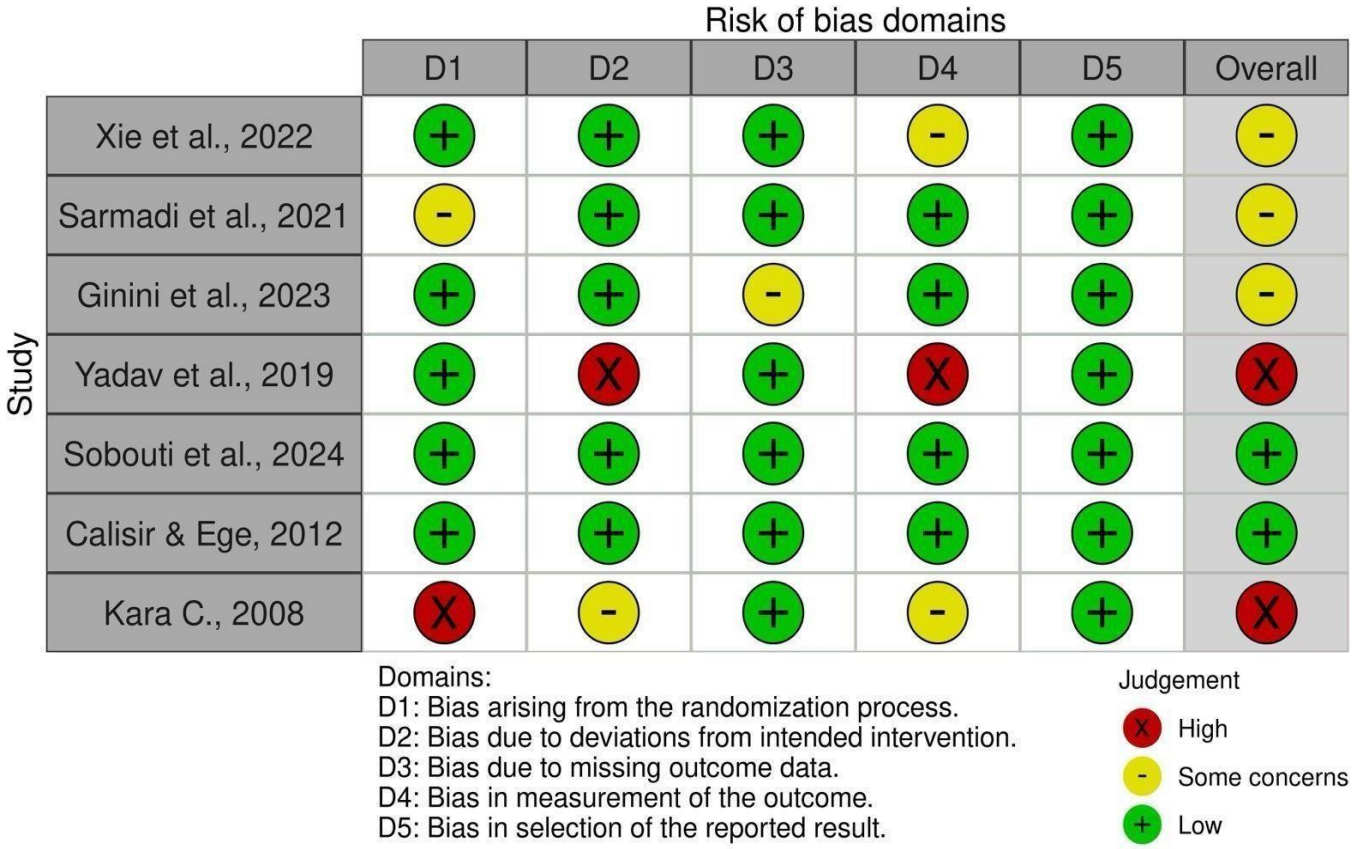


Figure 2. RoB 2 Domain-Specific Risk of Bias Judgments for Included Studies

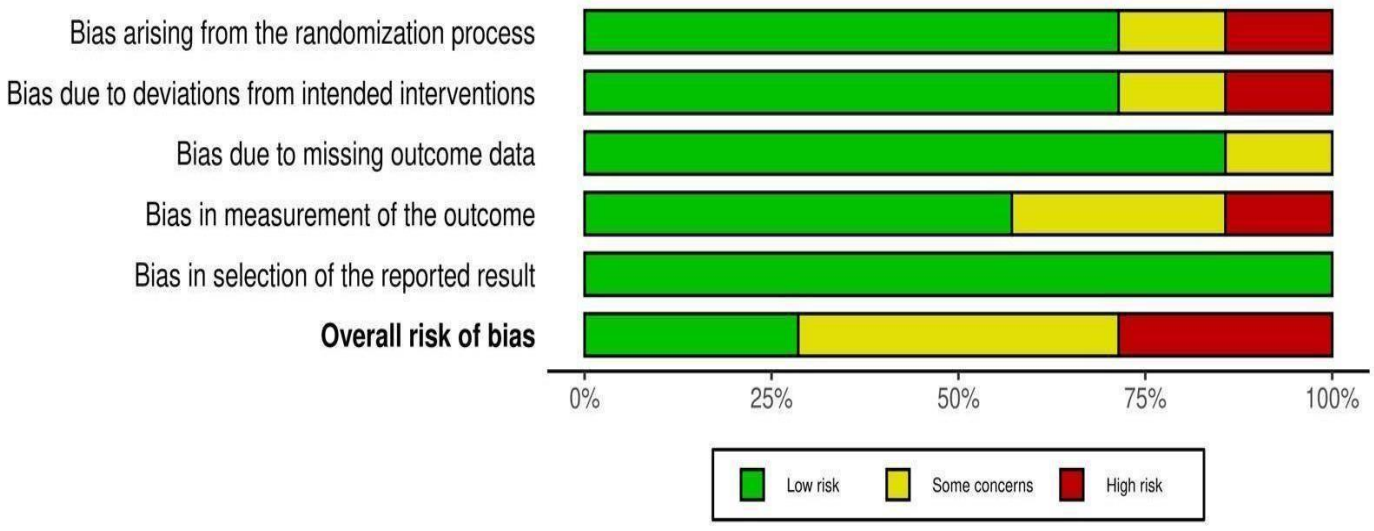


Figure 3. Proportion of Studies at Low Risk, Some Concerns, and High Risk by RoB 2 Domain and Overall

DISCUSSION

This systematic review investigated the clinical effectiveness of laser-assisted labial frenectomy against traditional scalpel surgery, relying purely upon evidence drawn from seven original studies [24]. The evaluation of outcomes measured operative time, bleeding during surgery, postoperative pain, healing, and patient satisfaction show consistent benefit from laser-assisted approaches, especially in pediatric groups.

Ling Xie et al. (2022) reported a randomized clinical trial in 34 age-matched pediatric subjects in the age range of 5–10 years, comparing a dual-wavelength laser treatment (Er:YAG and Nd:YAG) with the control scalpel treatment. The results show quite evidently that laser surgery decreases surgery time remarkably (mean: 224 ± 59 seconds) when compared with the scalpel treatment group (mean: 740 ± 168 seconds). Further, intraoperative pain as well as first postoperative day pain was lower in the laser group ($p < 0.05$). By the seventh postoperative day, no difference existed between groups in terms of pain experienced during mastication or speech. Healing was also consistently achieved, with a solitary instance of scar formation in the control scalpel group. The results indicate that dual-wavelength laser treatment not only streamlines the surgery in terms of efficiency but also minimizes immediate postoperative discomfort (Ling Xie et al., 2022).[18]

In a randomized and single-blinded study similar in design, Sarmadi et al. (2021) contrasted laser surgery with the use of a scalpel in a cohort of 40 patients. They noted a reduced intraoperative bleeding in the laser group by gauze weight (0.16 g for laser vs. 0.44 g for scalpel; $p < 0.05$). In addition, the laser group's mean procedure time was shorter (6.7 minutes vs. 10.5 minutes; $p < 0.01$). A larger wound size was noted postoperatively in the laser group, but this was no longer evident by the fifth postoperative day. Satisfaction was similarly high in both groups, showing that though the laser was better at intraoperative metrics, the overall experience was the same for both treatment modalities (Sarmadi et al., 2021).[19]

The research by Ginini et al. (2023) was centered on 49 pediatric cases (2–6 years of age), evaluating the effect of CO_2 laser versus scalpel on pain, satisfaction, and improvement according to the perceptions of parents. Though pain scores in the initial period of 72 hours were noticeably lower in the group treated with a scalpel (mean VAS: 3.6) when contrasted with the laser treatment group (mean VAS: 4.9), parents of laser-operated children were highly satisfied as well as perceived clinical improvement one month after surgery (mean satisfaction score: 9.1 vs. 7.2; $p < 0.001$). The findings emphasize the significance of long-term perceptions as well as functional outcomes in treatment evaluation. The eventual assessment by caregivers was in favor of laser treatment in terms of higher scores for aesthetics and functioning, even though initial pain was relatively more in the laser-operated cases (Ginini et al., 2023).[21]

The results of Yadav et al. (2019) supporting the clinical advantage of laser technology come from a cohort of 20 adult patients. The results showed a statistically significant decrease in intraoperative bleeding ($p = 0.016$), postoperative pain ($p = 0.016$), and the use of analgesics ($p = 0.008$) in the patients undergoing treatment with the use of Nd:YAG laser. The assessment of healing in the three-month follow-up, however, showed no difference between the two groups ($p = 0.095$), indicating that while laser treatment shows clear short-term advantages, the long-term outcomes of tissue healing are no better from those from traditional scalpel methods (Yadav et al., 2019).[20]

The Sobouti et al. (2024) randomized clinical trial stands out in its large sample size of 174 patients and its comparison of 445 nm versus 980 nm diode lasers against traditional scalpel surgery. The most optimal results were demonstrated by the 445 nm diode laser. Patients in this treatment arm showed lower pain scores post-surgery and at day seven ($p < 0.05$), in addition to quicker tissue healing on days 7 and 30. The laser treatment arms also revealed lower intraoperative bleeding and functional discomfort compared to the control scalpel arm (all $p < 0.05$). The 980 nm variant was also superior to the scalpel but inferior to the 445 nm variant. These results highlight the implication of laser wavelength in therapeutic outcomes, with shorter wavelengths bestowing better accuracy and hemostasis in soft tissue surgery (Sobouti et al., 2024).[22]

A valuable one is the split-mouth design study by Calisir and Ege (2018), wherein direct intra-patient comparison was made possible. Frenectomy was performed in forty patients on each side, one with the use of Nd:YAG laser and the other with the traditional use of a scalpel. The pain scores at the time of surgery as well as the first postoperative day were lower in the laser-operated side (mean VAS: 2.1 vs. 4.5; $p < 0.05$). Patients also experienced better chewing and speaking comfort in the laser-operated side.

The intra-subject design enhances the strength of the results by obviating the use of inter-individual variation, thus supporting the argument for laser use superior comfort (Calisir & Ege, 2018).[25]

Kara, in a comparative study, researched patient perception after labial frenectomy with Nd:YAG laser versus the use of the scalpel. Though the study did not include detailed VAS values, qualitative results showed more postoperative ease and higher postoperative satisfaction in the laser group. Improved hemostasis and a minimized necessity for suturing were also identified by the author as major advantages of laser surgery. Without numerical detail, the study adds valuable information regarding peripheral outcomes, which are important in reaching a conclusion regarding global treatment success, particularly in pediatric or anxious populations (Kara, 2008).[26]

Overall, the studies under review uniformly report shorter operating time, less bleeding during operation, and better immediate postoperative conditions resulting from laser-assisted frenectomy. Pain and functional discomfort are also lower in laser groups, most notably in the critical first few postoperative days. Though long-term outcomes tend to be the same with laser versus scalpel methods in many studies, patient and caretaker satisfaction is higher in laser groups. The wavelength, as well as the laser source itself, also affects the degree of clinical benefit noted, with preference shown for use of the 445 nm laser, as well as for the Nd:YAG laser, due to their accuracy and efficiency. It should be noted, however, that whereas the clear-cut short-term benefits are reported by some studies (i.e., Yadav et al., 2019[20]; Sarmadi et al., 2021[19]), they also show similar healing results following more protracted periods of follow-up. Thus, though laser methods produce better perioperative results, whether they are superior in the long term is in question and requires further longitudinal study. Economic considerations, too, as well as access to laser technology, may preclude extensive use, especially in low-resource environments.[28–38] However, the collective evidence favors the use of laser technology as a safe and in many cases preferred alternative in labial frenectomy when compared with scalpel surgery. Future research involving standardized outcomes, larger sample sizes, and longer follow-up periods will play a key role in cementing the position of laser-assisted methods in regular use in clinics.

CONCLUSION

Laser-assisted labial frenectomy can be considered a safe treatment with effective results in comparison with traditional scalpel surgery. It has definite advantages in terms of shorter operation time, minimal intraoperative bleeding, and diminished immediate postoperative discomfort. The advantages translate into better patient comfort in addition to quicker functional restoration. The overall patient satisfaction with laser methods is great, especially in cases involving children, where minimal distress is a key consideration. Healing results are similar between the two approaches, validating the clinical dependability of laser treatment. In conclusion, laser therapy improves the care in labial frenectomy and is a worthwhile addition in contemporary dental practice.

DECLARATIONS

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Availability of data and materials

The data will be available on reasonable request from the corresponding author.

Competing interests

The authors declare no conflict of interest.

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Author contribution

Conceptualization M.M.M.; G.M., G.C.; methodology, G.M.N. and M.P.S.; software, S.P.; and H.U.; formal analysis, S.Pa. and H.U.; investigation, M.P.S.; and S.P.; data curation, M.P.S. and G.M.N.; writing—original draft preparation, H.U., M.M.M.; writing—review and editing, S.Pa.; G.C. and G.M.; supervision, G.C. and G.M. All authors have read and agreed to the published version of the manuscript.

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RESEARCH ARTICLE

EVALUATION OF ANTIBACTERIAL, ANTIOXIDANT, AND CYTOTOXIC PROPERTIES OF GREEN SYNTHESISED ZINC OXIDE NANOPARTICLES FROM SANTALUM ALBUM: AN IN VITRO STUDY

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ABSTRACT

Background: Periodontal disease is a prevalent condition that requires effective treatments to manage inflammation and bacterial infections. Traditional therapies face challenges such as antibiotic resistance and limited efficacy. Green synthesised nanoparticles (NPs), particularly Zinc oxide nanoparticles (ZnO NPs), offer a promising alternative due to their unique biological activities. This study aims to green-synthesize ZnO NPs using *Santalum album (S. album)* leaf extract and evaluate their antibacterial, antioxidant, and cytotoxic properties, for application in the management of severe forms of periodontal disease

Materials and Methods: ZnO NPs were synthesized by mixing *S. album* leaf extract with Zinc acetate solution, followed by heating and centrifugation. The antibacterial activity was assessed using the Kirby-Bauer disk diffusion method against *Staphylococcus aureus (S. aureus)*, *Streptococcus mutans (S. mutans)*, *Escherichia coli (E. coli)*, and *Klebsiella pneumoniae (K. pneumoniae)*. Antioxidant activity was evaluated using DPPH and H_2O_2 scavenging assays. Cytotoxicity was determined through the zebrafish embryo model.

Results: The sZnO NPs demonstrated significant antimicrobial activity against *S. aureus, S. mutans, E. coli*, and *K. pneumoniae* with the highest activity against *S. mutans*. Their antioxidant activity was comparable to Vitamin C and Vitamin E, suggesting potential for further exploration. Zebrafish embryos treated with sZnO NPs showed proper development and comparable mortality rates with the control, indicating biocompatibility.

Conclusion: The green synthesised sZnO NPs have shown good antibacterial and antioxidant properties with low cytotoxic activity, allowing their further exploration for clinical application in the form of local targeted drug delivery agents for the treatment of severe forms of periodontal disease.

Keywords: Periodontal disease; nanoparticles; zinc oxide; green synthesis; antibacterial; antioxidant; cytotoxicity

1.INTRODUCTION

Periodontal disease has remained one of the major oral diseases affecting millions of individuals worldwide. According to the assessment of the status of the National Oral Health Policy in India in 2015, it is one of the two common oral diseases, with 89.2% of individuals affected by the disease in the age group of 35-44 years.[1] It is a chronic inflammatory disease affecting the supporting tissues of the teeth, that initiates in response to microbial plaque. The resultant host-microbial interaction leads to tissue destruction and the production of free radicles and reactive species, which further perpetuates the inflammatory process. Though various non-surgical treatment modalities have been explored, additional use of chemotherapeutics has been warranted in a few cases of hyper-responsive phenotypes. Oral and topical antimicrobials, local drug delivery, mouthwashes, and lozenges have been commonly used in the control of periopathogenic bacteria. With the emergence of super-resistant bacterial strains due to the imprudent use of systemic antibiotics and their associated side effects, alternative antibacterial agents are the need of the hour.[2] Moreover, the adjunctive use of antioxidants has also been shown to play a beneficial role in reinstating the tissues back to a state of health.

With the advances in biotechnology, nanodentistry has emerged as a promising solution for multiple facets of dental diagnosis and treatments. Nanomaterials, nanosensors, nanoimaging, and nanorobotics are aspects of nanotechnology that have been explored for application in the treatment of various periodontal diseases and conditions.[3-6] In particular, nanomaterials have been developed for application as local drug delivery agents, desensitizing solutions, mouthwashes, gum paints, etc[7]. Nanomaterials are particles ranging in size from 1-100 nm that are manipulated at the nanoscale for functionalization.[8] They have been further classified into organic and inorganic nanoparticles (NPs). These inorganic NPs express different biologic properties like antibacterial, antioxidant, anti-inflammatory, anti-cancer, and anti-viral.[9]

Zinc oxide nanoparticles (ZnO NPs) are tiny semiconducting particles known for their distinctive electrical and optical characteristics due to a substantial bandgap of 3.4 electron volts (eV).[10-12] Their unique properties trigger photochemical reactions by causing electrons to transition from the valence band to the conduction band, setting off a sequence of photochemical processes that generate reactive oxygen species. It is these reactive compounds that are accountable for the antibacterial effects of ZnO NPs.[10,13] They have also been found to be extremely biocompatible. Their increased surface area to volume ratio and improved tunability of the size, morphology, and surface properties are also advantageous for local drug delivery applications.

Green synthesis of NPs has received tremendous attention in recent years as an environmentally sustainable substitute for traditional chemical approaches that often use hazardous chemicals, produce toxic byproducts, increase environmental temperatures, and need costly complex equipment. The green synthesis of NPs involves the use of natural sources such as plants, bacteria, and fungi extracts which are great sources of biomolecules that can act as reducing and stabilizing agents in the synthesis of NPs. NPs synthesised using this method have shown many advantages like effectiveness, safety, scalability, and sustainability.[14]

Various herbal extracts have been explored for the synthesis of NPs.[15,16] *Santalum album (S. album)*, commonly known as Indian sandalwood, is a tree native to India, Indonesia, and several other countries in Southeast Asia. It is well known for its fragrant wood which is used in perfumes, incense, and traditional medicines. The essential oil extracted from the wood of *S. album* has been used for various medicinal purposes such as treating skin disorders, respiratory problems, and digestive issues. Studies have also reported potential antibacterial, anti-inflammatory, and anti-oxidant properties from santalum album extracts.[17] The aim of the present study was to green synthesise ZnO NPs using *S. album* extract (sZnO NPs) and to assess its antibacterial, antioxidant, and cytotoxicity properties.

MATERIALS AND METHODS

The study was conducted at the Department of Biomaterials, Saveetha Dental College, Chennai. Approval for conduction of the study was obtained from the Institutional Scientific Review Board.

ZnO NPs synthesis using *S. album (sZnO NPs)*: *S. album leaves* were freshly procured from Andhra Pradesh, India, from the latitude of 11,99674 and longitude of 92,9909. The leaves were carefully rinsed with running tap water to eliminate any dirt and dust particles adhering to them. Subsequently, they were allowed to air dry in the shade until completely moisture-free. They were then finely pulverized using an electric grinder. ZnO NPs were synthesised using the wet chemical process. The *S. album* leaf extract was prepared. Ten grams of the dried leaf powder was mixed with 100 ml of distilled water in a 500ml conical flask. The mixture was agitated at a constant speed of 130 rpm and 37 °C for 1 day to obtain a homogenous mixture. The leaf extract was acquired from the mixture by filtering it with the Whatman No 1 filter paper. Subsequently, 100 ml of the leaf extract was then added to 100 ml of 1M Zinc acetate solution while maintaining the Ph at 8. The flask with the mixture was

heated at 80 C for 3 hours, which lead to the precipitation of white material. The precipitate was washed with ethanol and subjected to centrifugation at 6000 rpm. The resultant pellet was collected and heated at 200 °C for 3 hours to obtain the pure sZnO NPs sample for further testing.

Antibacterial assay: The antibiotic susceptibility was carried out towards four common human oral bacteria namely *Staphylococcus aureus (S. aureus)* MTCC-740, *Streptococcus mutans (S. mutans)* MTCC-890, *Escherichia coli (E. coli)* MTCC-443, and *Klebsiella pneumoniae (K. pneumoniae)* MTCC-109. The nutrient broth was prepared and subjected to the sterilization process. The bacteria were individually inoculated and incubated at 37 °C for 8 hours to obtain bacterial colonies. The Kirby-Bauer disk diffusion test was performed. Each bacterial sample was evenly inoculated on the surface of Muller-Hinton agar plates. Four plates were prepared, one plate for each bacteria. In each plate 5mm discs were added in the plates. Disc A was loaded with antibiotic control (10µg/ml ceftazidime + 10 µg/ml erythromycin), disc B was loaded with a low concentration of sample (10 µg/ml) and Disc C was loaded with a high concentration (20 µg/ml). All the plates were incubated at 37 °C for 24 hours and the zone of inhibition was recorded and analyzed.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay: The antioxidant activity of sZnO NPs was assessed using the DPPH assay. Vitamin C was used as the control. Initially, a stock solution of DPPH was prepared by dissolving 22 mg of DPPH in 100 ml of methanol, resulting in a filtered solution with an absorbance of approximately 0.892 at 517 nm. Various ratios of DPPH and sZnO NPs were combined, with a standard ratio of 3 ml of DPPH solution in 100 µL of methanol. Following a 30-minute incubation period, the absorbance of the solutions was measured at 517 nm, and the percentage of antioxidants was then calculated and graphed.

Hydrogen peroxide (H_2O_2) assay: The H_2O_2 scavenging potential of sZnO NPs was investigated using the traditional UV method at 230 nm. To evaluate this activity, a colorimetric approach was employed. In this method, the reaction between H_2O_2, phenol, and 4-aminoantipyrine in the presence of horseradish peroxidase generates a pink quinoneimine dye. The alteration in color was used as an indicator of scavenging activity. This calorimetric technique was applied to both a standard antioxidant, ascorbic acid, and various concentrations of sZnO NPs (control, 40, 60, 80, 100, and 120 µg/mL). After a 30-minute incubation period at room temperature, the absorbance of the solutions was measured at 504 nm.

***In vitro* cytotoxicity assessment of sZnO NPs on zebrafish embryos:** The zebrafish model was used to analyse the *in-vitro* toxicity of the synthesised sZnO NPs. the test group were cultured with different concentrations of sZnO NPs while the control group were cultured with saline. The sZnO NPs were diluted at different ratios from 25-200 ug/ml. Twenty five numbers of zebrafish embryos were selected for the analysis. The zebrafish were well managed and assessed at different time periods. The number of dead zebrafish was estimated and differentiated with control samples. The zebrafish embryos were maintained at individual trails at a standard temperature for the generation of organs. The embryos were observed under a microscope at 40x magnification every 24 hours. At every four hour time period, the viable and dead embryos, and fish were identified. The dead embryos and fish were discarded at every inspection to minimise suspension contamination. The mortality ratio was calculated for every 24 hours.

RESULTS

Antibacterial assay: On analysis of the antibacterial activity observed with sZnO NPs, the zone of inhibition increased in proportion with the concentration of the sZnO NPs while the zone of inhibition noted with the antibiotic standard was consistently lower than that noted with the low concentration of the sZnO NPs, for all the bacterial strains. The results are depicted in Fig 1, Fig 2, and Table 1. A statistically significant difference in the antibacterial activity of sZnO NPs in comparison to the antibiotic standard was observed for both *S.aureus* and *S.mutans*, with the most antibacterial activity by sZnO NPs noted against s.mutans at both low and high concentrations. However, a variability in response was noted between gram-positive and gram-negative strains highlighting the importance of cell wall composition in determining bacterial resistance or susceptibility to nanoparticles.

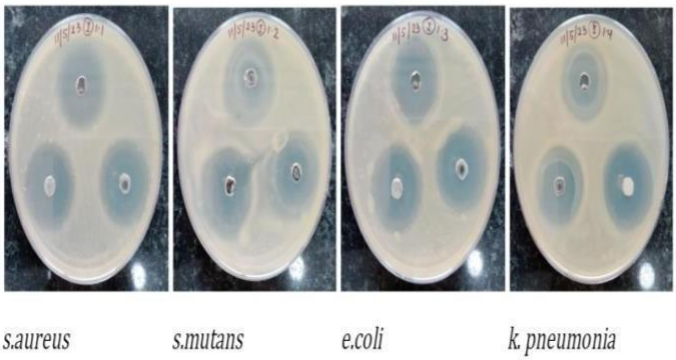


Figure 1. Antibacterial assay performed using sZnO NPs

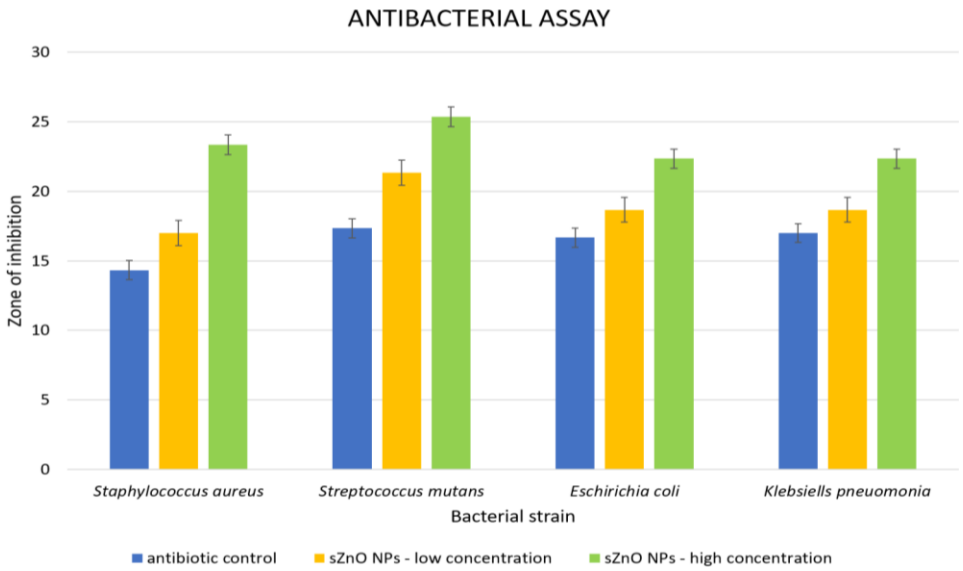


Figure 2. Comparison of the antibacterial activity of sZnO NPs with the antibiotic control

Table 1. Comparison of antibacterial activity of sZnO NPs and antibiotic control - one-way ANOVA

Strain	Concentration	N	Mean (Zone of Inhibition)	Standard Deviation	P Value
Staphylococcus Aureus	Antibiotic Standard	3	14.33	0.577	0.031*
	sZnO NPs - Low Concentration	3	17	1.000	0.001*
	sZnO NPs - High Concentration	3	23.33	1.154	0.000*
Streptococcus Mutans	Antibiotic Standard	3	17.33	1.527	0.042*
	sZnO NPs - Low Concentration	3	21.33	1.527	0.042*
	sZnO NPs - High Concentration	3	25.33	1.628	0.002*
Escherichia coli	Antibiotic Standard	3	16.66	1.423	0.315
	sZnO NPs - Low Concentration	3	18.66	1.652	0.059
	sZnO NPs -	3	22.33	1.715	0.009*

	High Concentration				
Klebsiella pneumoniae	Antibiotic Standard	3	17.00	2.000	0.647
	sZnO NPs - Low Concentration	3	18.66	2.081	0.186
	sZnO NPs - High Concentration	3	22.33	2.516	0.058

DPPH assay: The results of the DPPH assay, as depicted in Fig 3 and Table 2, demonstrate the antioxidant activity of sZnO NPs across varying concentrations, compared to Vitamin C (Vit C) as a standard. While both sZnO NPs and Vit C exhibited a concentration-dependent increase in DPPH radical scavenging activity with Vit C consistently showing higher activity at all tested concentrations, no statistically significant difference in the antioxidant activity was observed between the two samples. The results suggest that the antioxidant activity of sZnO NPs is comparable to Vit C. The observed trend highlights sZnO NPs potential as an antioxidant, albeit not as effective as the standard, Vit C. These findings could support the exploration of sZnO NPs as a complementary antioxidant agent for clinical applications.

Table 2. Comparison of antioxidant activity of sZnO NPs and vitamin C using the DPPH assay - unpaired t-test

Concentration	Sample	N	Mean (% Of Antioxidant Activity	Standard Deviation	P Value
2µg/ml	sZnO NPs	3	21.66	3.511	0.632
	Vit C	3	26.66	2.516	
4µg/ml	sZnO NPs	3	27	2.000	0.801
	Vit C	3	29.66	2.081	
6µg/ml	sZnO NPs	3	32.66	3.511	0.305
	Vit C	3	39.33	1.527	
8µg/ml	sZnO NPs	3	39.33	2.309	0.328
	Vit C	3	43.33	1.527	
10µg/ml	sZnO NPs	3	43.33	1.635	0.519
	Vit C	3	50.33	2.092	

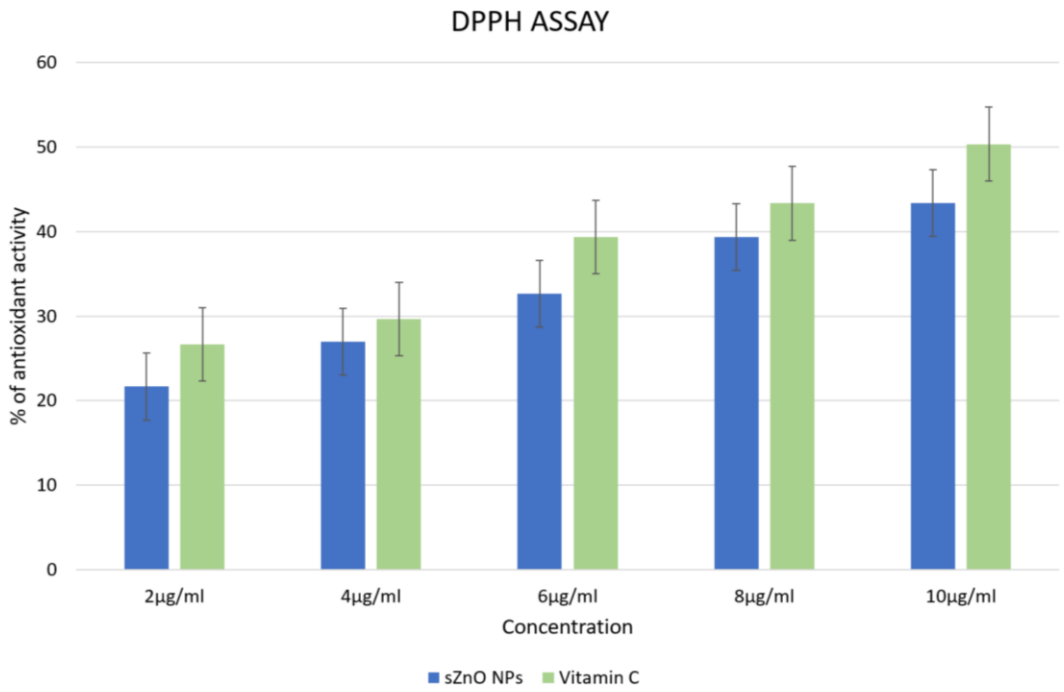


Figure 3. Antioxidant analysis of sZnO NPs using the DPPH assay

H_2O_2 assay: The results as depicted in Fig 4 and Table 3, indicate the antioxidant activity of sZnO NPs in comparison to Vitamin E (Vit E) across various concentrations. Both sZnO NPs and Vit E demonstrate a concentration-dependent increase in activity, as evidenced by the rising mean percentages of scavenging activity. However, Vit E consistently exhibits slightly higher activity than sZnO NPs at all tested concentrations, though there was no statistically significant difference between them at all time points. The standard deviations for both sZnO NPs and Vit E remain relatively low, suggesting consistency in the results. Overall, sZnO NPs demonstrate promising antioxidant activity, comparable to that of Vit E. These findings highlight the potential of sZnO NPs as an alternative or complementary antioxidant agent, warranting further exploration.

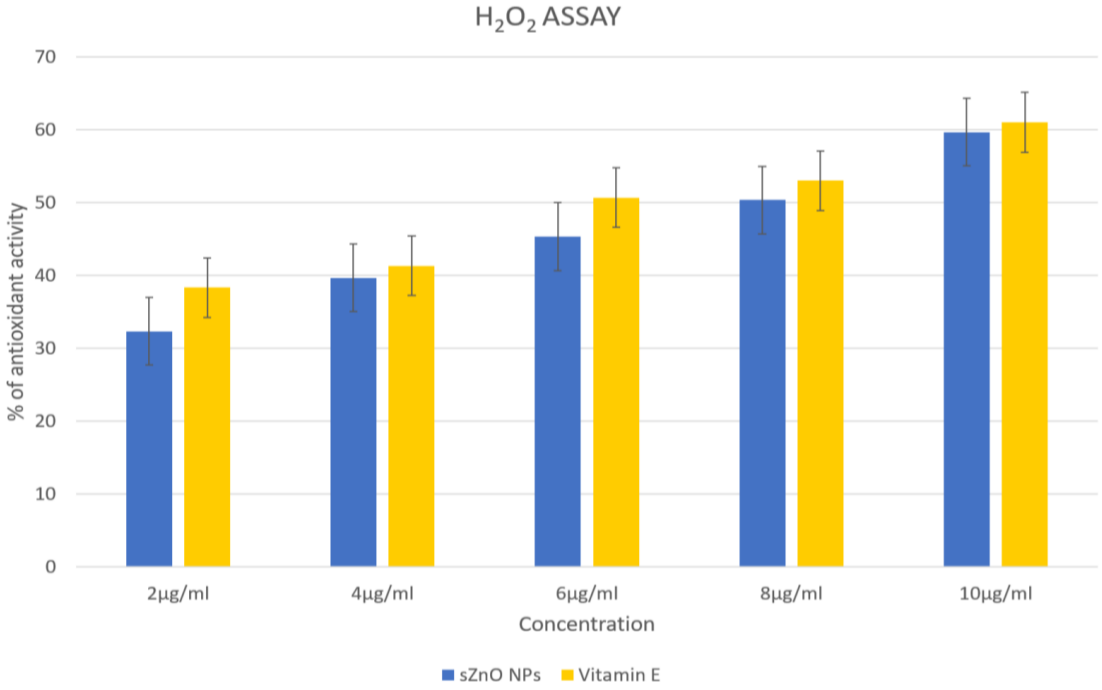


Figure 4. Antioxidant analysis of sZnO NPs using the H_2O_2 assay

Table 3. Comparison of antioxidant activity of sZnO NPs and vitamin E using the H_2O_2 assay - unpaired t test

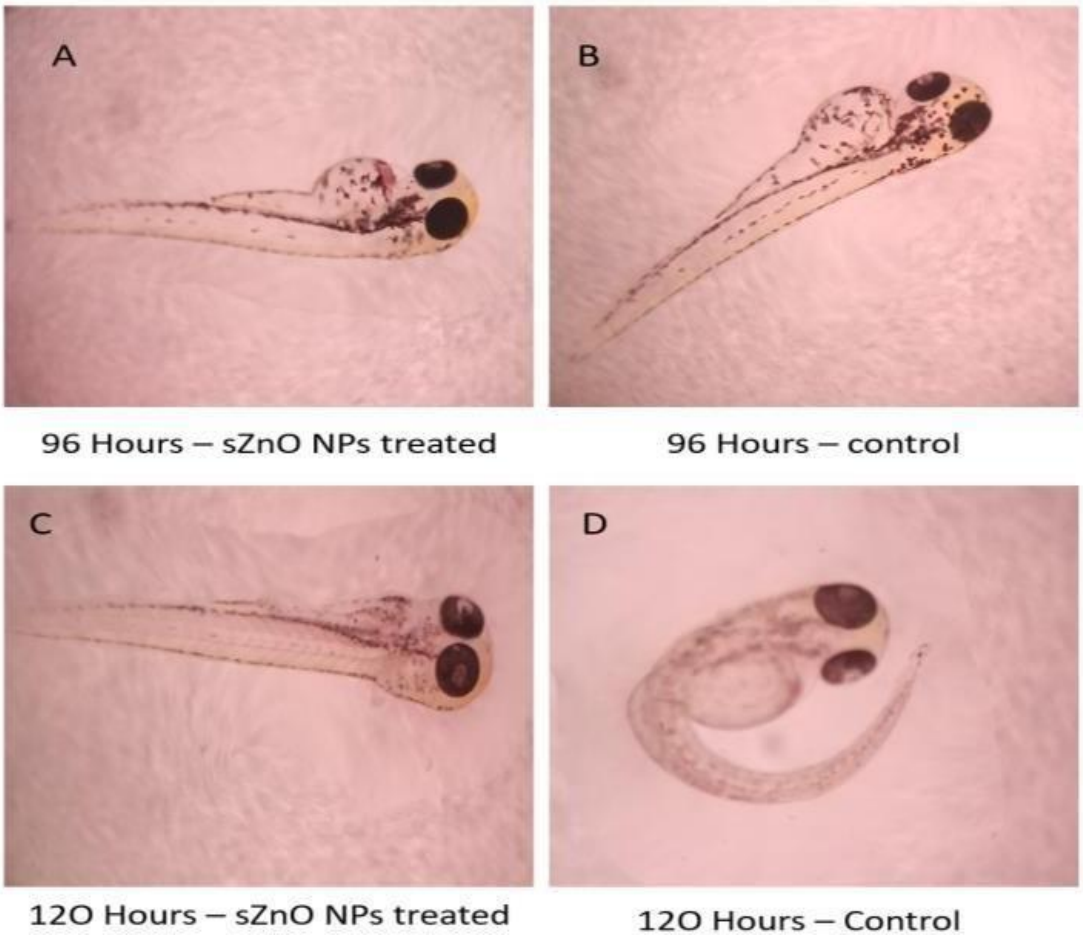
Concentration	Sample	N	Mean (% Of Antioxidant Activity)	Standard Deviation	P Value
2µg/Ml	sZnO NPs	3	32.33	4.041	0.655
	Vit E	3	38.33	3.055	
4µg/mL	sZnO NPs	3	39.66	2.081	0.812
	Vit E	3	41.33	2.516	
6µg/mL	sZnO NPs	3	45.33	1.527	0.519
	Vit E	3	50.66	2.081	
8µg/mL	sZnO NPs	3	50.33	0.577	0.057
	Vit E	3	53	2.645	
10µg/mL	sZnO NPs	3	59.66	2.081	0.801
	Vit E	3	61	2.000	

Cytotoxicity assay

The zebrafish embryos treated with sZnO NPs, as depicted in Fig 5, showed proper development of head, tail, eye, and internal organs at the correct time intervals, comparable to that of the control group, with several hatching and displaying various growth stages at 92 and 120 hours. The mortality trends observed in this study, as depicted in Fig 6 and Table 4, show that both the sZnO NPs and control groups show a consistent increase in mortality percentages, peaking at 120 hours, with sZnO NPs at 84.33% and the control at 85.66%.

This indicates a time- dependent factor influencing mortality, potentially linked to environmental conditions, experimental setup, or toxic progression. The close mortality rates at 120 hours suggest that sZnO NPs treatment does not significantly alter long- term outcomes compared to the control.

Furthermore, at all time points no statistically significant difference in mortality rate between the two groups was observed. This lack of significance implies that sZnO NPs does not introduce notable toxicity relative to the control, which proves that sZnO NPs is as biocompatible as the control.



Figur 5. Development of zebrafish embryos treated with sZnO NPs and control at various time points

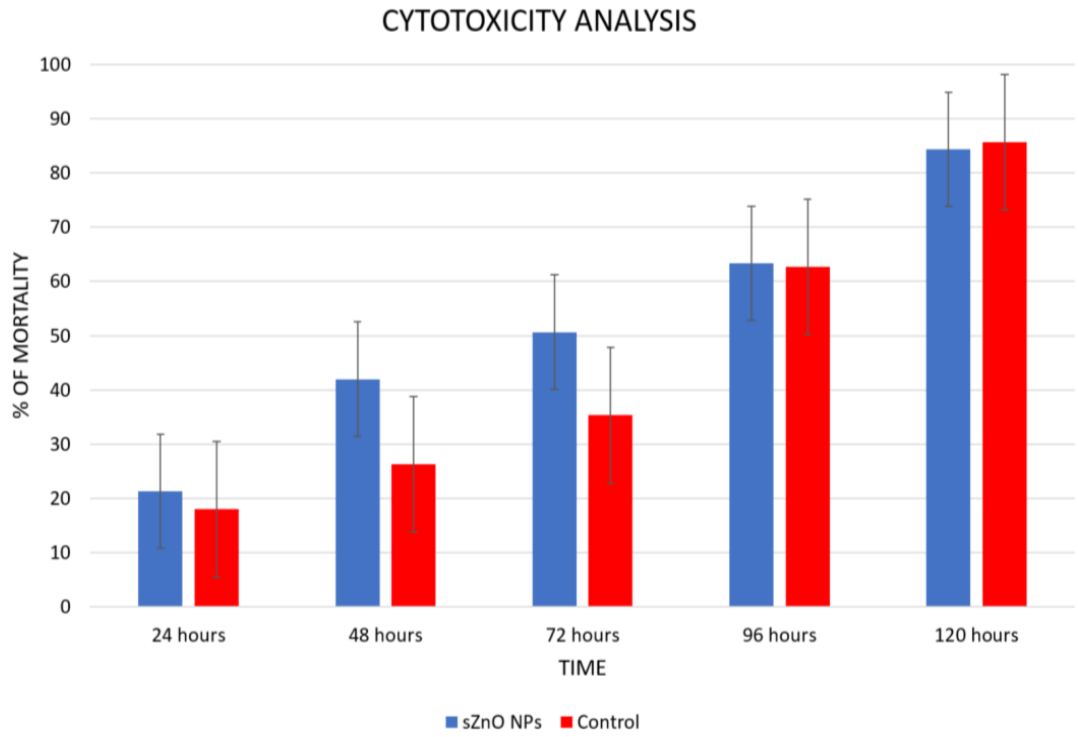


Figure 6. Cytotoxicity analysis of sZnO NPs observed in zebrafish embryos

Table 4. Comparison of cytotoxicity of sZnO NPs and control - unpaired t-test

Time	Sample	N	Mean (% Of Mortality)	Standard Deviation	P Value
24 HRS	sZnO NP	3	21.33	3.511	0.425
	Control	3	18.00	2.000	
48 HRS	sZnO NP	3	42.00	3.605	0.751
	Control	3	26.33	4.163	
72 HRS	sZnO NP	3	50.66	3.055	0.875
	Control	3	35.33	3.511	
96 HRS	sZnO NP	3	63.33	3.214	0.539
	Control	3	62.66	2.516	
120 HRS	sZnO NP	3	84.33	4.163	0.667
	Control	3	85.66	3.511	

DISCUSSION

Over the recent years, with the increase in life expectancy of the global population, there has been a greater emphasis on achieving enhanced quality of life. Periodontally healthy tissues improve the masticatory function, nutritional status, and esthetics of an individual. Though various mechanical therapies aim to remove the primary etiologic factors like plaque and calculus, complete eradication of the microbes from the intra-epithelial sources is not possible. They act as a source of reinfection, perpetually maintaining the tissues in a state of disease. In order to reduce the microbial load in the soft tissues, adjuvant antimicrobial therapy is often employed in the form of systemic or topical antimicrobials. However, due to the injudicious use of systemic antimicrobials, super-resistant bacterial strains have emerged causing aggressive forms of periodontal disease. Moreover, the inflammation-mediated free radical and reactive species generation has made the adjuvant use of antioxidants desirable. Hence multiple natural and synthetic sources are being explored to discover components with antimicrobial and antioxidant properties of which NPs have gained attention.

The antibacterial assay revealed that sZnO NPs exhibit a concentration-dependent antimicrobial effect, with higher concentrations consistently showing greater efficacy against the tested bacterial strains. This trend indicates that the sZnO NPs inhibitory activity likely increases with dosage, possibly due to enhanced interaction with bacterial cells or improved reactive oxygen species (ROS) generation at higher concentrations. For *S. aureus* and *S. mutans*, the antibacterial activity of sZnO NPs at both low and high concentrations significantly surpasses the antibiotic control, with statistically significant P-values ($P < 0.05$). In the case of *E. coli*, significant improvement is observed only at the high concentration of sZnO NPs, while for *K. pneumoniae*, the observed increase in inhibition zones at higher concentrations lacks statistical significance. These findings suggest that sZnO NPs are particularly effective against certain bacterial strains, such as *S. aureus* and *S. mutans*, and their efficacy increases with higher concentrations. However, differences in sensitivity across strains could be attributed to variations in cell wall structure or bacterial resistance mechanisms.

The gram-positive bacteria in this study, showed significant zones of inhibition for both low and high concentrations of sZnO NPs, exceeding the antibiotic control in both cases. This heightened susceptibility can be attributed to the simpler cell wall structure of gram-positive bacteria, which primarily consists of a thick peptidoglycan layer. The lack of an outer membrane may have enabled easier NPs penetration and interaction with cellular components, disrupting metabolic processes or inducing oxidative stress. In contrast, the gram-negative bacteria demonstrated relatively lower sensitivity to sZnO NPs. While *E. coli* showed significant inhibition at high concentrations, *K. pneumoniae* did not achieve statistically significant differences at either concentration. The reduced susceptibility of gram-negative bacteria can be linked to their more complex cell wall architecture, which includes an outer membrane rich in lipopolysaccharides, which may have acted as a protective barrier, limiting the penetration of nanoparticles and reducing their efficacy. These results highlight the potential of sZnO NPs as a broad-spectrum antimicrobial agent, though optimization may be necessary to enhance effectiveness against less responsive strains.

Studies have postulated various mechanisms leading to the antimicrobial activity of ZnO NPs.[18-21] It has been proposed that the positive charges from ZnO NPs are attracted to the negative charges in the peptidoglycan and lipoteichoic acid present in the cell membrane. This causes electron gradient development, along with a chelation reaction between the lipoteichoic acid and zinc ions. This destabilises the membrane potential, causing damage to the cell surface, cell morphology and the deoxyribonucleic acid (DNA) ultimately leading to cell rupture.[18,20,21] Its photocatalytic activity and strong oxidizing nature also lead to the formation of ROS which impairs protein synthesis and DNA replication.[22]

A recent study synthesised ZnO NPs using four different plant extracts and analysed their antibacterial and antifungal activity.[23] They observed that all samples exhibited antibacterial activity against the chosen bacteria except *Beta vulgaris-based* ZnO NPs that were ineffective against *S. aureus*. They concluded that green synthesised ZnO NPs were cost-effective and environment-friendly and must be further explored for their antimicrobial activity. Another study stated that ZnO NPs prepared using leaf aqueous extract of *Azadirachta indica (A. indica)* depicted increased antimicrobial activity in accordance with the increase in the concentration of the NPs.[24] This is similar to the findings of our study. They also stated that the ZnO NPs showed good antimicrobial activity which may be attributed to the increase in H_2O_2 concentration released from the surface of the NPs. ZnO NPs synthesized using *Aloe vera (A. vera)* were assessed for antimicrobial activity against *Staphylococcus epidermidis (S. epidermidis), K. pneumoniae, E. coli,* and fungi[25].

It was observed that the NPs were effective against *E. coli* and *Aspergillus niger* (*A. niger)* while the efficacy against other tested bacteria and fungi was less in comparison to the commercial antibiotic control. They postulated that the efficacy against gram-positive bacteria may have been less due to the presence of thick peptidoglycan layers of the cell membrane. This is in contrast to the results observed in our study.

The antioxidant analysis highlights the ability of sZnO NPs to exhibit antioxidant activity comparable to established standards like Vit C and Vit E across varying concentrations. Despite consistently showing slightly lower activity than both standards, the absence of statistically significant differences underscores the promise of sZnO NPs as viable alternatives or complementary agents in antioxidant applications. The results of this study are in accordance with another study that synthesized ZnO NPs mediated by *Coccinia abyssinica (C. abyssinica)* and showed slightly lower radical scavenging capacity than standard ascorbic acid at all concentrations, with an IC50 value of 127.74 µg/ml[26]. A recent study that analysed the antioxidant activity of ZnO NPs synthesised using *Pelargonium odoratissimum (P. odoratissiumum*) aqueous leaf extract, showed scavenging effect with an IC50 value of 28.11 ± 0.01 µg mL−1 compared to L-ascorbic acid. They stated that the comparable antioxidant activity to Vit C may be attributed to the bioactive constituents and higher phenolics and flavonoid content in *P. odoratissimum*.[27] Similar results of comparable antioxidant properties of green synthesised ZnO NPs to that of Vit C have been reported by recent research.[28-32]

The antioxidant potential exhibited by ZnO NPs can be explained through multiple mechanisms, leveraging their unique nanoscale properties and composition. They effectively scavenge free radicals, such as ROS, by donating electrons or hydrogen atoms to stabilize these harmful molecules, thus protecting cellular components from oxidative stress.[33] Additionally, ZnO NPs have been shown to enhance the activity of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which are critical for neutralizing ROS.[34] The zinc ions released by ZnO NPs also play a role in their antioxidant function, as these ions protect sulfhydryl groups in proteins and compete with redox-active metals, thereby reducing ROS generation.[35] Moreover, the small size and high surface area of ZnO NPs amplify their reactivity and improve their interaction with biological systems, enhancing their overall antioxidant capacity.[36] These mechanisms collectively highlight the potential of ZnO NPs in mitigating oxidative stress and their possible periodontal applications.

In relation to the cytotoxicity analysis of sZnO NPs on the zebrafish, it can be inferred that sZnO NP does not significantly alter mortality compared to the control under the given experimental conditions. The increasing trend in mortality for both groups might be influenced by external factors such as environmental conditions, depletion of resources, or inherent progression in the setup. These outcomes suggest that while sZnO NP does not appear to introduce significant toxicity compared to the control. Hence, we can conclude that sZnONPs are safe to be employed as antibacterial and antioxidant agents for periodontal applications. Similar results were observed in other studies that assessed the cytotoxicity of ZnO NPs developed through green synthesis.[37,38]

In summary, the green synthesised sZnO NPs exhibited good antibacterial activity against the chosen bacterial strains with greater zones of inhibition noted with gram-positive bacteria. The antioxidant activity of sZnO NPs increased proportional to the concentration of the NPs and was comparable to that of Vit C and Vit E. The cytotoxicity analysis showed a comparable mortality rate to that of the control, suggesting that the developed NPs did not induce cytotoxic activity on their own, other than that which occurred due to other experimental and environmental conditions. Hence, sZnO NPs warrant further exploration for their applicability as a local drug delivery agent with synergistic antibacterial and antioxidant activity. It's combined effect would allow for a more targeted effect that would also be more acceptable to the patient. The limitations of this study were that the synthesised sZnO NPs were not subjected to characterisation. Also, the biocompatibility could have been assessed with cell lines. Further, *in vitro* and *in vivo* studies can be conducted to study the antifungal, antiviral, and anti-inflammatory properties of the NPs and explore their clinical applicability in the management of severe forms of periodontal disease.

CONCLUSION

The study reveals that ZnO nanoparticles green synthesised from *S. album* exhibit good antibacterial and antioxidant activity. Moreover, they are safe for biomedical application which was proven by the comparable cytotoxicity observed with the control. These NPs are environmentally friendly and cost-effective, reducing the use of harsh chemicals and toxic byproducts. The findings suggest that further exploration of ZnO NPs antibacterial and antioxidant properties could lead to the development of new targeted local drug delivery applications for combating severe forms of periodontal diseases.

DECLARATIONS

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Conflicts of interest and financial disclosures

The authors declare no conflict of interest and there was no external source of funding

Ethical approval: Approval for the conduction of the study was obtained from the Institutional Scientific Review Board.

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REVIEW ARTICLE

IMPLANT IMPRESSION MAKING REVISITED

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ABSTRACT

.Digital impression technology has revolutionized implant dentistry by improving the planning, placement, and restoration of implants. Traditional conventional impression techniques were often inaccurate and uncomfortable. Intraoral scanners now provide precise 3D models for accurate surgical guides and prosthetic designs, integrating with CAD/CAM software for efficient workflows. The benefits include enhanced precision, reduced chair time, and improved patient comfort. Real-time feedback and seamless CAD/CAM integration minimize errors. Clinically, digital impressions support pre-surgical planning, custom guides, and follow-up care, improving implant success rates, especially with CBCT integration. Challenges include high costs, clinician training, and limitations in scanning certain areas. Environmental factors like moisture and large file management also pose difficulties. However, advancements in implant planning software and CAD/CAM technologies continue to improve efficiency. Future innovations in AI, machine learning, and cloud systems will further enhance diagnostic accuracy and accessibility, transforming implant dentistry and patient care.

Keywords: Digital Impression, Implants, Impression Techniques, Models

1.INTRODUCTION

INTRODUCTION

Implant dentistry offers solutions for missing teeth, providing functional, aesthetic, and durable outcomes. Success depends on precise planning, placement, and restoration, making accurate impressions essential for creating surgical guides, custom abutments, and prostheses. Traditional methods, involving silicone or alginate materials, often led to inaccuracies, discomfort, and inefficiencies. These limitations prompted the development of digital impression technologies, now the gold standard in implant dentistry.

History of Digital Impression Technology

Digital impression technology began in the early 1990s with scanning devices aimed at dental impressions. Early systems like CEREC (1985) were

bulky and inaccurate. Advancements in the 2000s, including Lava C.O.S. and iTero, improved precision and user-friendliness. Integration with implant planning and surgical guides, along with the advent of Cone Beam CT (CBCT), further refined digital workflows. Today, intraoral scanners are compact, fast, and highly accurate, seamlessly working with CAD/CAM and implant planning software for precise implant placement.[1]

Fundamentals of Digital Impressions in Implant Dentistry

Digital impressions in implant dentistry use advanced scanning technology to capture highly accurate 3D images of a patient's oral structures, eliminating the need for traditional impression materials. Intraoral scanners (IOS) capture detailed digital models of the teeth, gums, and oral cavity, which are then used for treatment planning, prosthesis design, and implant placement. This method provides superior accuracy, enabling precise implant planning and customization of restorations, reducing errors and improving fit. It also supports the creation of surgical guides for optimal implant placement. Digital impressions are faster, more comfortable, and less invasive than traditional methods, improving patient comfort and reducing the need for retakes. Additionally, they enhance communication among dental professionals and streamline the process, ensuring better collaboration and outcomes. Overall, digital impressions offer a more efficient, precise, and patient-friendly alternative to conventional techniques in implant dentistry.[1] Common scanning technologies include optical scanning (iTero, Trios), light reflectance (Planmeca Emerald), and laser triangulation (3Shape TRIOS, Carestream CS 3600). Many scanners combine these methods for improved precision and real-time feedback.[2]

Types of Digital Impression Systems

Digital impression systems vary by hardware, software, and clinical application.

- **Intraoral Scanners**: Handheld devices used for capturing digital impressions, such as iTero, Trios, and Planmeca Emerald. These scanners offer fast, accurate results with real-time feedback.
- **Chairside and Laboratory-Based Systems**: Chairside systems, like CEREC, are portable for in-office use, while laboratory systems (e.g., 3Shape D800) handle complex restorations.
- **Software Solutions**: CAD software (e.g., Exocad, 3Shape Dental System) designs restorations, while CAM software (e.g., Dental Wings CAM) facilitates manufacturing. Digital implant planning software (e.g., coDiagnostiX, BlueSkyPlan) aids in virtual implant placement.

Advantages of Digital Impressions in Implant Dentistry

- **Accuracy and Precision**: Digital impressions provide superior accuracy, essential for precise implant placement, custom restoration design, and surgical guides.[3,4]
- **Time Efficiency**: Faster scanning reduces chair time, enhances workflow, and allows for direct file transfers to labs, speeding up the overall process.
- **Patient Comfort**: No messy materials or bulky trays, making the procedure more comfortable, especially for patients with sensitive gag reflexes.
- **Reduction in Errors**: Real-time feedback allows for instant corrections, reducing distortion, voids, and errors.
- **Integration with Digital Workflow**: Direct connection to CAD/CAM systems streamlines design and manufacturing, reducing adjustments.
- **Enhanced Communication**: Instant sharing of digital files improves collaboration between clinicians and labs, ensuring better outcomes.

Clinical Applications of Digital Impressions in Implant Dentistry

1. **Pre-Surgical Planning**: Digital impressions create accurate 3D models that integrate with implant planning software and CBCT for precise placement and virtual simulations of implant surgeries.
2. **Surgical Guides**: Custom surgical guides, created using CAD/CAM, ensure accurate implant placement and reduce complications.
3. **Implant Prosthetics**: Digital impressions aid in designing custom restorations, such as crowns and bridges, and allow for rapid fabrication of temporary prosthetics.
4. **Follow-Up and Long-Term Monitoring**: Digital impressions track changes in oral anatomy, helping detect complications like bone loss early and facilitating prosthetic adjustments.

Challenges and Limitations of Digital Impressions in Implant Dentistry

- **Cost Considerations**: Intraoral scanners have a high upfront cost with ongoing maintenance and training expenses.
- **Learning Curve**: Clinicians must learn new scanning techniques and integrate digital
- impressions into existing workflows, which may disrupt efficiency initially.
- **Material and Technique Limitations**: Issues with scanning deep bites, soft tissue, or reflective prosthetic materials may require supplementary methods or traditional impressions.
- **Data Quality and Management**: Poor scanning technique or environmental factors can lead to incomplete scans and data loss, requiring rescans and causing treatment delays.
- **Intraoral Scanning Limitations**: Moisture, difficult-to-reach areas, and patient movement can affect scan accuracy, requiring extra time or specialized techniques.[2,3,4]

Digital Impressions and Implant Planning Software

Digital impressions, when integrated with implant planning software, improve the precision of implant placement. Popular systems like coDiagnostiX and BlueSkyPlan combine digital impressions with CBCT data, creating accurate 3D models and virtual treatment planning. Benefits include improved visualization, accurate placement, and minimized complications. Integration with CBCT provides detailed views of bone and soft tissue, enhancing implant planning and ensuring safe placement by identifying critical structures like nerves and sinuses.[5,6]

Digital Impressions and CAD/CAM Technology in Implant Dentistry

1. **Integration with CAD Software**: Digital impressions facilitate the design of precise custom restorations, including abutments and prostheses. CAD software allows real-time design modifications for optimal results.
2. **CAM (Computer-Aided Manufacturing)**: After design, CAM systems use milling or 3D printing to fabricate restorations, offering customization and high precision.
3. **Benefits of CAD/CAM Workflow**: Faster turnaround times, improved fit, fewer adjustments, enhanced aesthetics, and cost savings result from digital impression integration with CAD/CAM.[1,6,8]

Future Directions in Digital Impressions for Implant Dentistry

Advancements in technology are continuously transforming implant dentistry. Key trends shaping the future include:

- **Advances in Scanner Technology**: Future scanners will capture finer details, offer faster scans, and provide real-time feedback for improved accuracy.
- **Artificial Intelligence and Machine Learning**: AI will automate error correction, predict treatment outcomes, and enhance diagnostic capabilities.
- **Wearable and Mobile Devices**: Handheld scanners and smartphone integrations will make digital impressions more accessible in various settings.
- **Interoperability**: Unified systems will streamline workflows, allowing seamless data transfer between devices and software for improved efficiency.
- **Patient-Centered Innovations**: Future scanners will be more comfortable and affordable, benefiting both patients and practitioners.[1]

Comparison of Digital vs. Traditional Impressions in Implant Dentistry

Factor	Digital Impressions	Traditional Impressions
Accuracy	Higher accuracy, micron-level precision, no material distortion.	Prone to distortion and errors due to material handling and setting.
Time Efficiency	Faster procedure, immediate feedback, reduced re-impressions.	Slower procedure, material setting time, and risk of re-impressions.
Cost Effectiveness	High initial investment, but long-term savings in materials, labor, and fewer re-impressions.	Lower initial cost, but higher long-term costs due to material and labor needs.
Patient Experience	More comfortable, faster, reduces gag reflex and discomfort.	Often uncomfortable, longer procedure, more likely to induce gag reflex.

DISCUSSION

Much of the existing literature on digital impressions in implant dentistry stems from experimental studies, particularly in vitro research, which provides useful insights but has limited applicability to clinical settings. Only a few in vivo studies have been included, and these generally lack precise measurements of accuracy that would allow direct comparison with in vitro results. Therefore, additional in vivo studies with reliable outcome reporting are necessary to gain a better understanding of the accuracy of digital impressions in real-world clinical situations. When comparing conventional and digital impression techniques, available evidence indicates that digital impressions offer comparable accuracy for fixed partial prostheses. However, high-quality evidence supporting the preference of digital impressions over conventional methods for full-arch rehabilitation is still lacking, despite promising in vitro results. [10,11,12]

Regarding patient preference, all the reviews examined agree that patients prefer digital impression technology over traditional impressions.[8] Digital workflow enhances clinical efficiency in terms of impression time, patient preference, and time-saving (De Oliveira et al.,2020).[5] Intraoral scanning causes less discomfort for patients compared to traditional methods and improves the overall patient experience in terms of comfort(Bishti et al.,2021).[6] García-Gil et al. evaluated the efficiency, accuracy, and economic feasibility of digital impressions for implant-supported restorations. Their review of 27 studies showed similar passive fit outcomes between digital and conventional methods, with various factors influencing accuracy. Digital impressions are viable for single or adjacent implants, but further research is needed for full-arch restorations. In vitro data indicates that the accuracy of 3D digital impressions is comparable to that of conventional implant impressions, though more in vivo studies are required to assess accuracy in clinical practice.[3,7]

Studies suggest that the accuracy of digital impressions may decrease as the number of implants increases or the edentulous span widens due to errors in image stitching. However, the literature has not yet defined the exact threshold at which these errors become clinically significant.[9] Number of implants, scanning protocol, Intra-oral scanners and edentulous span affects accuracy of digital impressions but all provide clinically acceptable results, with only minor variations in precision.[10,11]

The scanning protocol, especially the starting point for scanning, plays a critical role in ensuring accuracy. Starting the scan from the restoration area may reduce distance and angular deviations, leading to better outcomes. Regarding implant angulation, most studies suggest that digital impressions are not significantly affected by different implant angulations, especially in controlled laboratory conditions. However, in vivo factors such as patient movement and saliva may influence accuracy, and further studies are necessary to assess these variables.[12]

CONCLUSION

Digital impressions in implant dentistry offer key benefits such as enhanced accuracy, improved patient comfort, and increased efficiency. Technologies like intraoral scanners integrated with CAD/CAM systems enable precise implant placement and restoration design, resulting in better-fitting restorations and more predictable outcomes. The elimination of traditional impression materials reduces patient discomfort, minimizes gagging, and shortens chair time. A fully digital workflow, integrating CBCT data and real-time communication with labs, streamlines treatment. Challenges include high initial costs, the need for ongoing training, and limitations in certain scans. In conclusion, although digital impressions show potential in implant dentistry, further in vivo research is needed to clarify the impact of factors such as implant depth and angulation, scanning protocols, and overall impression accuracy. These studies will be crucial for determining the best workflows for digital implant dentistry.

DECLARATIONS

Conflicts of interest and financial disclosures

The author declares that he has no conflict percent and there was no external source of funding for the research in question.

Ethical Approval

Not applicable as the present article was a review article.

Informed Consent

Not Applicable as it is a review article and doesn't require any patient's involvement

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RESEARCH ARTICLE

EVALUATION OF THE ANTI-CARIOGENIC PROPERTY OF *STEVIA REBAUDIANA* - BASED NOVEL LOZENGES

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ABSTRACT

Background & Aim: Dental caries is a widespread oral health issue predominantly caused by *Streptococcus mutans*, a bacterium known for its ability to form biofilms on tooth surfaces. The use of non-cariogenic sweeteners with potential therapeutic benefits offers a promising strategy for caries prevention. *Stevia rebaudiana*, a natural plant-based sweetener, has shown antimicrobial properties, but its application in child-friendly formulations such as lozenges remains underexplored. This study aimed to develop lozenges using *Stevia rebaudiana* and evaluate their antibacterial and antibiofilm efficacy against *Streptococcus mutans*, comparing their activity to commercial sugar-free alternatives.

Methods: Lozenges were prepared using *Stevia rebaudiana* extract, palm sugar, ginger, lemon, and water. Extracted molar teeth were inoculated with *S. mutans* prepared in Brain Heart Infusion (BHI) broth. After incubation, treatments included natural sugar-free (control), commercial sugar-free green, and Stevia lozenges. The biofilm biomass was quantified using crystal violet staining, and viable bacterial counts were assessed through colony-forming unit (CFU) analysis after sonication and plating on BHI agar.

Results: The Stevia lozenges demonstrated a significant reduction in *S. mutans* viability and biofilm formation. CFU analysis showed 20 CFU/ml for both Stevia lozenge and sugar-free green treatments, in contrast to 1000 CFU/ml for the natural sugar-free control. Biofilm quantification supported these findings, indicating lower biomass in treated samples.

Conclusion: Lozenges formulated with *Stevia rebaudiana* effectively inhibited *S. mutans* growth and biofilm formation, showing comparable efficacy to commercial sugar-free products. This study highlights the potential of Stevia lozenges as a natural, non-cariogenic, and child-friendly alternative with therapeutic applications in oral health care.

Keywords: Anti-cariogenic, antimicrobial, biofilm, dental caries, lozenges

INTRODUCTION

Stevia rebaudiana, a species native to tropical South America, is renowned for its medicinal properties and natural sweetness[1]. This plant contains sweet compounds like rebaudioside A and stevioside, making it a potential aid in managing conditions such as diabetes, obesity, and hypertension. With the rise in demand for natural sweeteners, *Stevia rebaudiana*, containing stevioside and other glycosides, has gained popularity as a low-calorie alternative to sugar, particularly beneficial for diabetic individuals[2].

Stevia rebaudiana Bertoni leaves extract has been found to possess significant anti-cariogenic properties. This is attributed to its ability to combat the growth of cariogenic bacteria such as Lactobacillus acidophilus and Streptococcus mutans, which are known to be responsible for the development and progression of dental caries by forming biofilms on the surfaces of teeth[3,4]. Stevia extract contains various bioactive compounds, including flavonoids, alkaloids, saponins, sterols, and tannins, that have been shown to inhibit the growth of these bacteria, thereby providing a natural alternative for preventing dental caries[5]. Moreover, Stevia extract's antimicrobial properties have been found to extend to reducing bacterial load, biofilm formation, and maintaining oral pH levels, further supporting its anti-cariogenic potential[6].

This implies that Stevia extract can prevent the colonization and growth of cariogenic bacteria on the surface of the teeth, thus reducing the risk of dental caries. Additionally, Stevia extract is a safe and effective alternative to synthetic antimicrobial agents, making it an ideal choice for those seeking natural alternatives to prevent dental caries. Furthermore, Stevia's ability to reduce bacterial load, biofilm formation, and act as an anti-inflammatory agent contributes to its anti-cariogenic potential[7]. In vitro studies have demonstrated that *Stevia rebaudiana* ethanolic extract inhibits the acidogenic potential and formation of insoluble polysaccharides in Streptococcus mutans, further supporting its anti-cariogenic effects[8]. Overall, *Stevia rebaudiana* emerges as a promising option for oral health, offering benefits beyond just its sweetening properties. Therefore, this study aims to evaluate the anti-cariogenic property of a novel lozenge formulation containing *Stevia rebaudiana* extract. The study will utilize in vitro assays to assess the antimicrobial activity of the lozenges against common cariogenic bacteria, including Streptococcus mutans.

MATERIALS AND METHODS

Preparation of Lozenges

Ingredients needed to make lozenges include water, lemon, ginger, palm sugar, and *Stevia rebaudiana* leaves or powder. Palm sugar and water are cooked over a low flame until the sugar melts and the mixture takes on a liquid consistency. For flavor and medicinal benefits, lemon and ginger paste are added. Stevia powder is then added while they are thoroughly mixed. Further heating produces a paste-like consistency that is dried out and molded into appealing forms for kids to consume.

Treatment of Molar teeth with S. mutans

Bacterial inoculum in Brain Heart Infusion broth was prepared with 0.5 McFarland standard. And distributed in bottles. The teeth were put in the bacterial suspension and allowed to adhere to the teeth for 4 hours with frequency supplementation of media. Centrifugation was used to separate the planktonic cultures, which were then floated in Brain Heart Infusion broth after being spun at 2700 relative centrifugal force (RCF), for 10 minutes at 25 °C.

Quantification of Biofilm Biomass

After being fixed with 200 L of methanol at 25 °C for 30 minutes, biofilms were allowed to dry. About 250 µL of a 0.01% crystal violet solution was used to stain the biofilms for 15 minutes at 25 °C. The control solution contained 0.01% crystal violet. The wells were twice washed in 250 µL of sterile distilled water and then allowed to air dry at 25 °C. After a 30-minute static incubation period at 25 °C, 200 µL of a 30% acetic acid solution was added to each well, and the absorbance (AU) was measured at 570 nm.

Antibiofilm Activity

Based on colony-forming units (CFU), viable microbial colony counts were calculated. To spread the bacteria in the biofilm, 200 µL of sterile PBS was poured into each well, and the wells were then sonicated for 30 min. 10µL of the serially diluted scattered biofilms were observed on Brain Heart Infusion agar.

RESULTS

In our study, treatment with the extract has significantly decreased the bacterial viable count indicating the inhibition of growth of *S.mutans* and biofilm synthesis. The colony count of *S. mutans* was assessed following treatment of tooth samples with different sweetening agents: sugar-free green (positive control), natural sugar-free (negative control), and plant-based lozenges containing *Stevia rebaudiana* extract. The composition of each sample is given in **Table 1**

Table 1 shows the exact composition of the samples used in our study

Treatment	Type	Key Components	Role in Study
Natural Sugar-Free (Aquazyl Isomalt Lozenges Sugar Free Mint)	Commercial product	Sorbitol, erythritol, natural flavorings	Negative control – does not contain known antimicrobial agents
Sugar-Free Green (Eugica Coff Herbal Lozenges Sugar Free)	Commercial product	Stevia, xylitol, herbal extracts	Positive control – marketed for oral health, known anti-cariogenic ingredients
Stevia Lozenges	Experimental product	*Stevia rebaudiana* extract, lemon, ginger, palm sugar	Test sample – combines natural sweeteners with plant-based antimicrobial agents

Upon exposure to *S. mutans* inoculum prepared in Brain Heart Infusion (BHI) broth, the treated teeth showed varying levels of bacterial adherence and biofilm formation. After incubation and quantification procedures, a significant reduction in viable colony-forming units (CFU/ml) was observed in samples treated with Stevia lozenges and sugar-free green, as compared to the control (natural sugar-free):

- **Natural sugar-free (Control):** 1000 CFU/ml
- **Sugar-free green:** 20 CFU/ml
- **Stevia lozenges:** 20 CFU/ml
- The antimicrobial efficacy of *Stevia rebaudiana* lozenges against *Streptococcus mutans* was evaluated using a broth microdilution method, and the Minimum Inhibitory Concentration (MIC) was determined by monitoring turbidity as an indicator of bacterial growth. The results are depicted in **Figure 1**, which shows a clear dose-dependent reduction in bacterial turbidity with increasing concentrations of *Stevia rebaudiana* lozenge extract.

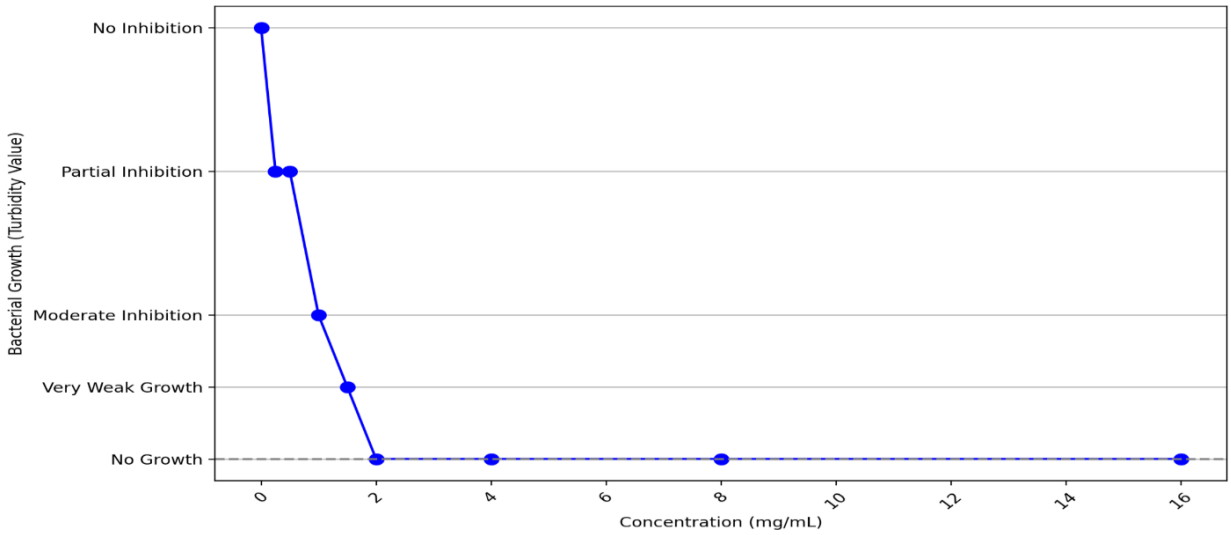


Figure 1 shows the bacterial growth in the teeth treated with prepared lozenges from *Stevia rebaudiana* against *S. mutans* at various concentrations

At 0 mg/mL (control), *S. mutans* exhibited uninhibited growth, corresponding to the highest turbidity values on the graph, indicating the absence of antibacterial activity in the absence of the lozenge extract. Partial inhibition of bacterial growth was observed at concentrations between 0.5 and 1 mg/mL, as seen by a modest reduction in turbidity. A further decrease in bacterial turbidity was recorded at 1.5 mg/mL, suggesting moderate inhibition. Notably, at 2 mg/mL, the bacterial growth was significantly reduced, with minimal turbidity values, indicating a near-complete inhibition of *S. mutans* growth.
Complete inhibition was observed at concentrations of 4 mg/mL and above (4, 8, and 16 mg/mL), where the turbidity values plateaued near zero, suggesting no observable bacterial growth. These concentrations are considered bacteriostatic or bactericidal under the test conditions. Thus, the Minimum Inhibitory Concentration (MIC) of *Stevia rebaudiana* lozenges against *S. mutans* was determined to be 2 mg/mL, with complete inhibition achieved at ≥4 mg/mL. These findings support the potent antibacterial properties of Stevia-based lozenges and their applicability in oral health care, particularly for the prevention of dental caries caused by *S. mutans*.
Quantification of biofilm biomass using crystal violet staining revealed a marked decrease in biofilm accumulation in Stevia lozenge-treated samples. Absorbance readings at 570 nm confirmed reduced staining intensity, suggesting lower biofilm density. The use of 30% acetic acid to solubilize the dye enabled accurate spectrophotometric measurement of biofilm mass. Additionally, viable counts following sonication and plating on BHI agar further supported the anti-adhesive and antibiofilm activity of the Stevia-based formulation. The reduction in CFU/ml in Stevia-treated samples was consistent across all replicates, demonstrating reproducibility and reliability.
The data indicate that Stevia rebaudiana lozenges significantly inhibit the growth and biofilm formation of *S. mutans*, with results comparable to the commercially available sugar-free green product. The natural sugar-free control, lacking antimicrobial components, showed the highest CFU count and densest biofilm, reinforcing the importance of incorporating bioactive plant extracts in oral formulations. These findings illustrate the potential of Stevia lozenges as a functional, non-cariogenic sweetener with significant anti-*S. mutans* activity, suggesting a promising role in the prevention of dental biofilm-associated diseases.

DISCUSSION

Dental caries remains one of the most prevalent chronic diseases worldwide, largely due to the activity of *Streptococcus mutans*, a bacterium well known for its capacity to adhere to tooth surfaces, form biofilms, and ferment dietary sugars into acids that demineralize enamel. This biofilm-mediated pathogenesis is strongly influenced by the type and frequency of carbohydrate intake, with sucrose being particularly cariogenic.[9] In this context, the need for non-cariogenic sweeteners with therapeutic properties is more pressing than ever. Our study demonstrates that lozenges made from *Stevia rebaudiana* extract significantly reduce both bacterial viability and biofilm formation by *S. mutans*, providing a potential dual-function oral health product—offering both sweetness and protection.

The lozenges formulated with *Stevia rebaudiana* showed a dramatic reduction in *S. mutans* colony-forming units (20 CFU/ml) compared to the control (natural sugar-free; 1000 CFU/ml). This represents a three-log reduction in bacterial load, highlighting the potent antibacterial activity of the Stevia-based lozenges. Interestingly, this efficacy was on par with a leading commercially available sugar-free green product, positioning Stevia lozenges as a competitive and potentially superior natural alternative. The observed reduction in biofilm biomass, as indicated by reduced crystal violet absorbance at 570 nm, supports the antibacterial findings and suggests that Stevia lozenges not only inhibit bacterial growth but also impair biofilm development. This is consistent with previous studies where Stevia reduces biofilm formation in *S.mutans* and *C.albicans* by decreasing the expression of genes such as gtfB and gbpB, which are crucial for EPS synthesis and thus demonstrating superior suppression compared to sucrose.[10,11] This is a critical feature, as the biofilm matrix plays a central role in protecting bacteria from host immune responses and antimicrobial agents. By disrupting biofilm formation, Stevia-based lozenges can directly influence caries development at an early stage.

Previous research has explored the antimicrobial properties of *Stevia rebaudiana* in various forms. and reported that aqueous Stevia extracts could inhibit *S. mutans* growth and reduce acidogenic potential. However, most of these studies relied on extract solutions or powders rather than edible formulations.[12-15] The current study extends these findings by incorporating the Stevia extract into a lozenge form that is suitable for pediatric and general use, improving both palatability and compliance. Moreover, prior work has primarily focused on in vitro inhibition zones or metabolic assays, while our study utilized a quantitative biofilm model involving real tooth surfaces, mimicking in vivo conditions more closely.[16-18]

This methodological approach enhances the translational value of the findings and offers a practical framework for future product development.

The mechanism by which *Stevia rebaudiana* exerts its antibiofilm effects is not yet fully elucidated, but it is hypothesized that compounds such as stevioside and rebaudioside A possess antimicrobial properties that interfere with bacterial adhesion, quorum sensing, and metabolic pathways.[19,20]

These glycosides may alter the surface properties of enamel or interact with bacterial cell membranes, leading to reduced colonization and impaired biofilm integrity.[21-23] In addition to Stevia, the lozenge formulation in this study included lemon and ginger—both of which possess known antibacterial and antioxidant properties. Lemon provides citric acid, which can modulate pH and disrupt bacterial metabolism, while ginger contains gingerol, a compound with proven antimicrobial action. These components likely exert a synergistic effect, amplifying the antibacterial activity of the formulation without compromising taste or safety. The novelty of this study lies in the development of a natural, Stevia-based lozenge formulation specifically designed to be both therapeutic and child-friendly. Unlike synthetic sweeteners, which have raised concerns over metabolic effects and long-term safety, Stevia offers a natural and safer alternative that not only replaces sugar but actively contributes to oral health. The fact that the lozenges performed as well as a top-tier commercial sugar-free product underscores their potential value.

Furthermore, the study's reproducibility across replicates enhances the credibility of the results and suggests potential for scalability in commercial production. As dental caries prevention moves toward integrative and preventive care, functional formulations like Stevia lozenges could play an important role in public health strategies, especially in pediatric populations and individuals with high caries risk.

Limitations and Future Directions

While the results of this study are promising, several limitations must be acknowledged. As the experiments were conducted under in vitro conditions, the findings may not fully reflect the complex dynamics of the oral environment in vivo. Therefore, future studies should include clinical or in vivo evaluations to confirm the real-world efficacy of the Stevia lozenges. Additionally, long-term assessments are necessary to understand the effects of regular lozenge use on the overall oral microbiome, ensuring that beneficial bacteria are not adversely impacted. Consumer acceptability, especially among children, should also be explored through sensory evaluation and taste preference studies to optimize palatability. Further research into the formulation's stability, storage conditions, and shelf life will be essential for commercial development. Finally, isolating and characterizing the specific bioactive compounds in *Stevia rebaudiana* responsible for the observed antibiofilm activity could enable the refinement and enhancement of the formulation for greater therapeutic benefit.

CONCLUSION

This study provides compelling evidence that *Stevia rebaudiana*-based lozenges significantly reduce *Streptococcus mutans* viability and biofilm formation, with efficacy comparable to established sugar-free products. The combination of natural ingredients with proven health benefits highlights the potential for such formulations to serve as effective, safe, and consumer-friendly alternatives in oral care, especially in the pediatric segment. This positions Stevia lozenges not only as a non-cariogenic sweetening solution but also as a novel, bioactive preventive strategy against dental caries.

DECLARATIONS

Ethical approval and consent to participate

Not Applicable

Availability of data and material

All data generated or analyzed during this study are included in the published article

Competing interest

The authors declare that there are no competing interest.

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PERCEPTION OF SAFETY: A SURVEY ON THE RISKS OF VAPING, HOOKAH, AND CIGARETTE CONSUMPTION

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ABSTRACT

Background: People are increasingly selective about alternative smoking techniques involving vaping and hookah because they consider these methods safer than conventional smoking. Many people continue to believe incorrect things about the medical hazards linked to these smoking methods.

Objective:A research study analyzed how people perceive health risks from both vaping, hookah, and cigarette usage among the surveyed population.

Methods:A survey with structured questions was conducted among 170 participants aged 18 or older. The evaluation instrument assessed how survey participants perceived the safety levels of vaping, hookah, and cigarette smoking. An analysis of the survey data was conducted to identify patterns and elements affecting perceived risk.

Results:A significant number of participants considered vaping, as well as hookah, to cause less harm than traditional cigarettes. Moreover, many people believed e-cigarettes, along with hookah devices, operate by creating chemicals in smaller amounts and lead to decreased health threats in the long run. Risk perception variations linked with age progressions, education completion degrees, and active smoking tendency were found to be significant by analysis.

Conclusion:A wide range of individuals continue to believe that vaping and hookah carry less health risk when compared to cigarette smoking, even though medical research demonstrates otherwise. Public health programs should strive to dismantle faulty beliefs about these products because this initiative could decrease the number of smoking-related health consequences.

Keywords: Cancer, Hookah, Nicotine, Perception, Vaping

1.INTRODUCTION

Many lives across India suffer from tobacco consumption, which creates substantial health risks, including cancer, that add significantly to the national disease burden[1]. All tobacco consumption forms, including cigarettes, bidis, smokeless products,

hookahs, and the new trend of vapes, lead to severe health consequences [2]. The Indian population shows significant tobacco use despite raising awareness, so many people proceed to develop fatal diseases like cancer [3].

The data shows that tobacco use exists among 28.6% of Indian adults. The adult population comprises more than one quarter of individuals who use tobacco, despite a reduction from previous years. The people of India use tobacco in smoking products as well as non-tobacco smokeless substances. The non-smoking tobacco products, such as gutka, khaini, and betel quid with tobacco, reach about 21% of the adult population throughout India. Such products exist in Indian culture due to deep-rooted tradition, even though research demonstrates their harm to health [4]. Tobacco smoking affects 10.7% of adults in India who use either conventional cigarettes or cheaper bidis tobacco products.

Studies show that the tobacco use problem also affects younger individuals. A proportion of 2% among young individuals between the ages of 10 and 14 smoke actively, but boys exhibit higher smoking rates than girls in this demographic group [5]. Early tobacco use initiation exhibits significant health risks for long-term addiction and develops chronic diseases, which becomes a concerning vital trend [6].

Hookah, along with vaping, has recently become trendy among young Indians who primarily live in cities [7]. Even though hookah enthusiasts might consider it a safe social activity, it actually creates additional health risks [8]. Using a hookah for one hour will expose users to dangerous smoke levels equivalent to smoking two entire packs of cigarettes. The health risks from hookah pipes become worse because of their carbon monoxide and heavy metal emissions. The Indian government implemented a ban on vapes, called e-cigarettes, in 2019 because of health risks and their increasing attraction for teens [9]. The ban has not been able to stop underground sources and online markets that sell these products.

Tobacco consumption leads to the dire outcome of cancer development because of its immediate effects on human health. Based on population demographics, smoking tobacco leads to 27% to 40% of all cancer diagnoses in India. Tobacco usage leads to cancer development among 48.7% of male patients and 16.5% of female patients [10]. People in India develop oral cancer, together with lung cancer, throat cancer, and esophageal cancer, as the primary forms of tobacco-related cancers. As a result, India contributes more than 83,000 cases out of the 120,000 instances of oral cancer that stem from smokeless tobacco and areca nut consumption worldwide [11].

The number of tobacco-related diseases keeps increasing which will lead to an expected rise in India's cancer burden from 1.39 million cases in 2020 to 1.57 million by 2025 [12]. The current increase in cancer cases is directly linked to the persistence of tobacco use, combined with insufficient education campaigns and insufficient stop-smoking programs and weak regulatory measures in different areas. Although COTPA and pictorial warnings have promoted more awareness their enforcement across India maintains an inconsistent distribution.

Strong tobacco control needs to combine regulatory enforcement with community-based awareness delivery and available de-addiction help along with specific prevention measures directed at youth populations [13]. People must understand the life-threatening medical problems which cigarettes along with smokeless tobacco and hookah devices and vapes can trigger. The elimination of combustion through vaping does not stop the delivery of harmful chemicals together with nicotine which makes vaping unsafe for non-smokers and teens.

Tobacco consumption through all its traditional and modern variants in India remains an acute health hazard. The direct connection to cancer, particularly oral and lung cancers, underscores the urgency of tobacco control initiatives. The fight against tobacco addiction together with cancer devastation in India requires stronger health regulations with educational programs that lead to accessible support systems for implementation in upcoming years.

Methodology

Study Design

A descriptive cross-sectional study was conducted to assess the knowledge, attitudes, and practices (KAP) related to the perceived risks and safety of vaping, hookah, and cigarette use among dental students and interns.

Study Setting and Participants

The study was carried out online, targeting undergraduate students (from the second year onward) and interns. A convenience sampling method was employed, resulting in 170 participants being included.

Inclusion and Exclusion Criteria

Students eligible for participation included those enrolled in the Bachelor of Dental Surgery (BDS) program from the second year onward and interns currently undergoing clinical training. Participants

who were absent during the data collection period or declined to provide informed consent were excluded from the study.

Data Collection Instrument

Data were collected using a structured, self-administered questionnaire specifically developed for this study. The questionnaire comprised sections addressing three domains:

Knowledge: Awareness of the health effects and chemical components of vaping, hookah, and cigarette products.

Attitude: Perceptions regarding the safety, acceptability, and social implications associated with these substances.

Practice: Self-reported use, frequency, and behavioral patterns associated with the consumption of tobacco and nicotine products.

The instrument was pretested on a small group of students to ensure clarity and validity. The feedback obtained was used to make minor modifications before its final deployment.

Data Collection Procedure

Participants were briefed on the study's purpose, and informed consent was obtained prior to their involvement. The questionnaire was distributed in print or through a secure online platform, ensuring voluntary participation and confidentiality throughout the process.

Data Analysis

Responses were compiled and analyzed using statistical software. Descriptive statistics were used to summarize demographic information and KAP responses. Associations between participant characteristics and KAP scores were examined using appropriate statistical tests, including chi-square and t-tests where applicable. Based on predefined benchmarks, a scoring rubric was developed to quantify knowledge, attitude, and practice levels.

Ethical Considerations

The Institutional Ethics Committee approved the study. Participation was entirely voluntary, and all responses were maintained in strict anonymity. No personal identifiers were collected.

Results

The analysis included 170 participants, with a gender distribution as follows: 70 (41.18%) participants were female, and 100 (58.82%) were male [Figure 1].

Male = 100
Female = 70

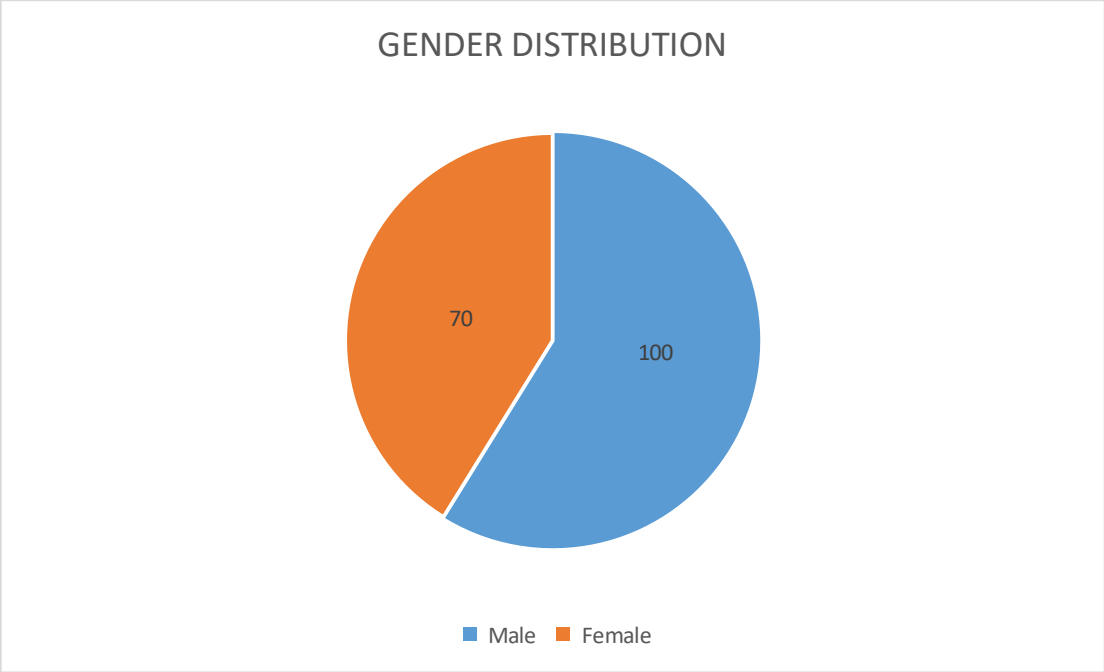


Figure 1. Gender Distribution

Knowledge Section

Is vaping completely risk free as compared to cigarettes- Out of all participants who took the survey 136 people correctly identified that vaping poses risks above zero compared to cigarette smoking yet 34 gave incorrect answers to the question. The study results show that 80% of participants realized vaping has risks beyond smoking while 20% held misleading views about its safety level.
whether hookahs contain nicotine- answer of whether hookahs contain nicotine was provided by 125 participants but 45 participants believe that hookahs don't contain nicotine . Among those interviewed 73.5% correctly identified hookahs contain nicotine while 26.5% either lacked knowledge or wrong information about this fact.

extended vaping leads to cancer development- The majority of 156 people correctly identified that extended vaping leads to cancer development but 14 respondents said that vaping doesn't cause cancer . The research findings show that people comprehended the risk of developing cancer from sustained vaping because 91.4% of participants acknowledged this danger while only 8.6% demonstrated misunderstanding.

"Passive smoking (second-hand smoke) can affect health?"- The survey about "Passive smoking (second-hand smoke) can affect health?" showed that 112 people were aware of health hazards but 58 people didn't know the effects of passive smoking . A total of 65.9% participants understood the health risks from passive smoking but 34.1% respondents lacked this knowledge.
whether nicotine could lead to addiction - Out of 170 participants the question about whether nicotine could lead to addiction received 120 correct answers and 50 believed that nicotine doesn't cause any form of addiction. Research findings show that 70.6% of participants realized nicotine is addictive yet 29.4% of respondents were unaware of this fact.
"**Are e-cigarettes FDA approved for smoking cessation?**"- For the question regarding "Are e-cigarettes FDA approved for smoking cessation?" a total of 106 participants provided correct answers but 64 participants displayed incorrect responses. Among those surveyed 37.6% displayed confusion about e-cigarettes being unapproved for smoking cessation by the FDA which contrasts with the 62.4% who understood the fact correctly.
Regarding hookah smoke compared to cigarette smoke- 141 participants were aware of the fact that hookah delivers more smoke whereas 29 people provided an incorrect response. The data indicates hookah delivers more smoke because 83% of respondents understood this fact while 17% did not receive this information.

Table 1: Knowledge assessment of participants

Questions asked	Answered - YES	Answered - NO
Is vaping completely risk-free compared to smoking cigarettes?	136	34
Do hookahs contain nicotine?	125	45
Prolonged vaping can cause: cancer	156	14
Does passive smoking (second-hand smoke) affect health?	112	58
Can nicotine be addictive?	120	50
Are e-cigarettes FDA-approved for smoking cessation?	106	64
Does hookah smoking deliver more smoke than cigarettes?	141	29

Attitude Section

vaping carries less risks than traditional cigarette smoking: The polled individuals expressed their agreement rates as follows: 10 (5.4%) strongly disagreed, while 19 (10.3%) disagreed and 29 (15.7%) stated neutrality, 70 (37.8%) agreed, followed by 42 (22.7%) who strongly agreed.

occasional hookah smoking is safe and not addictive: The respondents gave this statement their opinions 12 (6.5%) strongly disagreed; 15 (8.1%) disagreed; 24 (13.0%) stayed neutral; 78 (42.2%) agreed; and 41 (22.2%) strongly agreed.

Table 2. Attitude assessment participants

Question Text	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
I believe vaping is less harmful than smoking cigarettes.	10	19	29	70	42
Occasional hookah smoking is safe and not addictive.	12	15	24	78	41
Cigarette smoking by healthcare professionals sets a poor example for patients.	9	24	13	72	52
I feel peer pressure influences people to start vaping or smoking.	8	21	30	73	38
Dental professionals have a responsibility to discourage tobacco use.	4	18	23	83	42
Vaping is socially more acceptable among youth than cigarette smoking.	13	15	38	69	35
I would feel uncomfortable advising a patient to quit if I use tobacco myself.	7	15	26	67	55
Anti-tobacco awareness campaigns influence people's smoking behavior.	12	19	23	75	41

Cigarette smoking by healthcare professionals sets a poor example for patients: 9 participants (5.29%) strongly disagree , 24 participants (14.11%) disagreed with this statement, 13 participants (7.64%) were neutral with the question, 72 participants (42.35%) agreed , 52 participants (30.58%) strongly agreed.

Peer influences in vaping or smoking initiation: eight participants (4.3%) strongly disagreed with the statement, followed by 21 (11.4%) who disagreed while thirty (16.2%) remained neutral, 73 (39.5%) agreed with it, and 38 (20.5%) strongly agreed.

Dental professionals have a responsibility to discourage tobacco use: 4 respondents (2.35%) strongly disagreed,18 participants (10.58%) disagreed with the question, 23 (13.52%) were neutral , 83 (48.82%) agreed with the question, and 42 (24.70%) strongly agreed .

Vaping is socially more acceptable among youth than cigarette smoking: 13 participants (7.64) strongly disagree , 15 participants (8.82%) disagreed , 38 (22.35%) were neutral , 69 participants (40.58%) agreed where as 35 (20.58%) strongly agree.

When it comes to tobacco use in patients I advise on quitting: the results showed that seven respondents (3.8%) strongly disagreed, fifteen (8.1%) disagreed, twenty-six (14.1%) remained neutral, sixty-seven (36.4%) agreed and finally fifty-five (29.7%) strongly agreed with this statement.

"**Anti-tobacco awareness campaigns influence people's smoking behavior":** 12 participants (6.5%) strongly disagreed, 19 (10.3%) disagreed, 23 (12.4%) kept a neutral stance, 75 (40.5%) agreed, and 41 (22.2%) strongly agreed.

Practice Section

Have you ever tried cigarettes? Out of all participants, 31 people (18.2%) attempted cigarette use, but 139 (81.8%) had no experience with them.

Do you currently use any tobacco products? The survey revealed that 32 participants (18.8%) were currently using tobacco products among the total 170 respondents. Additionally, 138 participants (81.2%) did not use tobacco products.

Have you ever tried vaping (e-cigarettes) - If asked if they had ever tried vaping e-cigarettes through the survey 38 participants (21.6%) spoke yes but 132 participants (78.4%) reported no.

Have you used a hookah/shisha in the past 6 months - Out of the total participants, 29 individuals (16.5%) presently used hookah/shisha yet 141 (83.5%) disclaimed such usage in the last six months.

Do you read health warnings on cigarette/vape packaging - The Healthy Choices Adolescents Conduct Survey revealed that 125 participants (67.6%) regularly pay attention to health warnings on cigarette and vape packaging while 29 (15.7%) read them occasionally and 16 (8.7%) failed to do so.
Have you advised a peer to quit smoking or vaping - Among participants 134 (78.8%) reported having suggested former classmates stop smoking or vaping while 28 people (16.5%) stated they had not made such recommendations and 8 individuals (4.7%) remained undecided.
Do you believe you could confidently counsel a patient on tobacco risks - Among the respondents who were asked if they believed they could provide confident patient counseling on tobacco risks, 120 (70.2%) answered positively and 32 (18.7%) answered negatively while 18 (10.5%) were unsure.
Have you participated in any tobacco awareness campaigns or workshops - A total of 130 respondents (76.0%) reported taking part in tobacco awareness campaigns or workshops followed by 29 individuals (17.0%) who said no and 11 participants (6.4%) who were unsure.

Table 3. Practice assessment of participants

Question Text	Yes	No	Always	Sometimes	Never	Not sure
Have you ever tried cigarettes?	31	139	_	_	_	_
Do you currently use any tobacco products?	32	138	_	_	_	_
Have you ever tried vaping (e-cigarettes)?	38	132	_	_	_	_
Have you used a hookah/shisha in the past 6 months?	29	141	_	_	_	_
Do you read health warnings on cigarette/vape packaging?	_	_	125	29	16	_
Have you advised a peer to quit smoking or vaping?	134	28	_	_	_	8
Do you believe you could confidently counsel a patient on tobacco risks?	120	32	_	_	_	18
Have you participated in any tobacco awareness campaigns or workshops?	130	29	_	_	_	11

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Table 4. Constituents of hookah, cigarettes and vapes

Feature/Substance	Hookah (Hukkah)	Cigarettes	Vapes(E-cigarettes)
Nicotine	Yes (in tobacco)	Yes (main addictive component)	Often present (varies by product)
Tar	Yes (from tobacco combustion)	Yes	No (no combustion, but some harmful chemicals)
Carbon Monoxide (CO)	High (from charcoal)	Moderate (from burning tobacco)	Low or none, unless defective
Heavy Metals	Yes (from charcoal & pipe)	Yes (from tobacco and paper)	Yes (from heating coil: lead, nickel, etc.)
Carcinogens	Yes	Yes (over 70 known carcinogens)	Some (formaldehyde, acrolein may form when heated)
Other Chemicals	Glycerin, molasses, flavoring, PAHs	Over 7,000 chemicals (e.g., benzene, ammonia)	PG, VG, flavoring agents, additives
Flavors	Yes	Sometimes (menthol, clove, etc.)	Yes (wide variety: fruit, mint, candy, etc.)
Addiction potential	High (due to nicotine + social appeal)	Very High	Moderate to High (depends on nicotine level)
Smoke/Vapor Volume	Very high (denser and longer sessions)	Moderate	High (especially with sub-ohm devices)
Immediate Effects	Dizziness, headache, increased heart rate	Same as hookah; also shortness of breath	Cough, throat irritation, dry mouth
Long-Term Health Risks	Heart/lung disease, cancer, CO poisoning	Cancer, heart disease, stroke, lung damage	Unknown (still being studied), but risk exists

DISCUSSION

Tobacco consumption in India remains a significant public health concern, contributing to a substantial number of deaths annually.

Tobacco-Related Deaths in India - Annual Deaths: Tobacco use is responsible for over 1 million deaths each year in India [14]. Cancer Burden: Oral cancer is the third most common cancer in India, with over 77,000 new cases diagnosed in 2012. Studies estimate over five deaths per hour due to oral cancer in the country [15]. Tuberculosis and Beedi Smoking: Smoking beedis has been linked to 200,000 deaths caused by tuberculosis in India [16].

The study establishes vital findings about how people understand the safety risks between vaping and hookah compared to cigarettes [17].

The majority of participants admitted traditional cigarettes were risky yet numerous others believed vaping and hookah carried reduced health risks. Research indicates vaping receives global acceptance as a safer option though scientists continue to show that

e-cigarettes contribute to cardiovascular harm and respiratory issues [18]. Many participants failed to recognize the dangers in occasional hookah smoking because they unknowingly believed the myths that filtered hookah smoke is better than cigarette smoke despite evidence that demonstrates the same or worse toxic compounds in both substances [19].

Studies revealed that peer relationships proved to greatly impact subject behaviors [20]. The majority of participants acknowledged that peer pressure strongly affects the initiation of both smoking and vaping practices throughout the youth population [21]. Social dynamics show that acceptance from peers determines adolescent risky behavior choices. Research findings are encouraging because participants showed support for helping peers stop using tobacco by delivering advice while displaying consistent reading habits of package warnings.

Some healthcare professionals who use tobacco products risk encountering obstacles when counseling patients since they should act as modeling examples to the public. Many students have taken part in tobacco awareness education yet false beliefs about e-cigarettes and hookah demonstrate deficiencies in today's prevention approach [22].

Research data shows that antismoking campaigns achieved successful reduction of cigarette stigma yet e-cigarettes and hookahs have not received similar attention. Future academic interventions must address these alternative tobacco products while debunking safety myths and teaching healthcare students to become better tobacco life advocates.

CONCLUSION

This research delivers crucial knowledge about how individuals perceive and act regarding vaping along with hookah and cigarette use. The majority of individuals recognized broad hazards related to tobacco yet there were multiple widespread misinterpretations about hookah and vaping safety. Research subjects saw vaping as providing reduced risk than cigarette smoking while they considered hookah use risk-free yet understanding about its dangers remains essential. Most of the participants showed they wanted to avoid peer-to-peer tobacco dissemination and patient tobacco prevention while actively taking part in tobacco education activities.

Some healthcare workers who use tobacco products demonstrated inconsistent competence when it came to providing smoking cessation counseling. The positive effects of awareness initiatives toward tobacco education have been observed yet confusion about newer tobacco usage patterns persists. Healthcare providers must receive targeted education along with ongoing professional training for policy measures to assist patients and healthcare staff in developing tobacco-free lifestyles through better education of these practices.

DECLARATIONS

Ethical approval and consent to participate

Not Applicable

Availability of data and material

All data generated or analyzed during this study are included in the published article.

Competing interest

The authors declare that there are no competing interests.

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RESEARCH ARTICLE

FULL MOUTH REHABILITATION OF TURNER AND MISSIRLIAN CATEGORY I-WORN-OUT DENTITION - A CASE REPORT WITH SURGICAL, FUNCTIONAL AND ESTHETIC SUCCESS

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Severely worn-out dentition presents significant functional, aesthetic, and structural challenges, often impairing mastication, speech, and overall quality of life of the individual. This case report describes the full-mouth rehabilitation of a 58-year-old male patient diagnosed with Turner and Missirlian Category I wear, characterized by generalized attrition, loss of posterior support, and reduced vertical dimension of occlusion (VDO). A comprehensive treatment approach was adopted, integrating endodontic, periodontal, and prosthodontic interventions to restore function, stability, and aesthetics. The treatment plan involved a multidisciplinary approach, beginning with post and core restorations to reinforce structurally compromised teeth. Full-mouth surgical crown lengthening was performed to enhance retention for full-coverage crowns and optimize occlusal function. Prosthetic rehabilitation included fixed porcelain-fused-to-metal crowns supported by root canal-treated teeth and cast partial denture for posterior edentulous areas. The VDO was re-established using provisional restorations, ensuring patient adaptation before the final prosthesis placement. The patient was monitored over a one-year follow-up period, demonstrating improved function, aesthetics, and occlusal stability. This case highlights the importance of a systematic and evidence-based approach in managing severe tooth wear. By employing a structured treatment protocol, including gradual VDO increase, crown lengthening for improved retention, and the integration of fixed and removable prostheses, a predictable and long-term outcome was achieved. The rehabilitation successfully restored oral function and aesthetics while preserving remaining tooth structure, significantly enhancing the patient's quality of life.

Keywords: Attrition; Cast posts; Crown lengthening; Full Mouth Rehabilitation; Worn-out dentition; Vertical dimension of occlusion.

INTRODUCTION

Critically worn-out dentition is considered a debilitating condition from both functional and esthetic perspective of an individual which severely effects one's quality of life. In-vivo studies have suggested that the natural enamel undergoes a wear of about 0.3 mm in approximately 10 years, the excessive wear, leading to tooth structure loss results in deranged masticatory power, and speech impairment affecting the oral health and eventually the overall health of the individual[1]. Excessive wear can result in pathologies involving the pulp, occlusal derangement, impaired function and esthetics. In addition to hypersensitivity of teeth, one may even experience a compromised facial appearance due to reduced vertical dimensions of occlusion (VDO)[2], elicit higher propensity towards tooth fractures, and if not intervened, it may even lead to temporomandibular joint disorders and myofascial pain. The condition, thus, gradually results in a multitude of challenges owing to functional and aesthetic limitations. The masticatory impairment results in dietary restrictions, often leading to insufficient nutrition. Impaired speech, negatively impacts social interactions and well-being. The temporomandibular (TMJ) disorders may culminate in chronic pain, which further exacerbates discomfort and curb one's day-to-day activities.

The worn-out teeth may occur as a result of plethora of phenomenon, such as attrition, erosion, abrasion and even parafunctional habits such as bruxism and less frequently, congenital anomalies[3–6]. Attrition results from improper tooth-to-tooth contact due to conditions such as malocclusion or lack of posterior tooth support. Dental erosion is caused by enamel or dentin dissolution which frequently occurs in patients with acidic reflex, patients who often consume acidic foods and beverages. Abrasion occurs as a result of deranged mechanical forces due to injudicious tooth brushing techniques, and extensive use of highly abrasive toothpastes. The habit of bruxism has been significantly linked to extensive tooth-wear[7]. This habit can be diurnal or nocturnal, which may be triggered by occlusal interferences[8].

In addition, infrequent causes of tooth wear include chewing of tobacco, pipe smoking, biting objects like pen or pencil or holding them between the teeth. These less frequent habits are known to be associated with emotional stress. Moreover, the congenital anomalies have also been implicated as a causative factor for the accelerated wear of tooth structure. In conditions such as amelogenesis imperfecta, and dentinogenesis imperfecta, the calcified structures of teeth, namely, the enamel, and dentin soften, become friable, and when exposed to the oral cavity, they are subjected to rapid wear[9].

Preventive strategies play a pivotal role in preventing the initiation or slowing down the progression of extensive wear. Periodic oral health check-ups aid early diagnosis and prompt treatment[10]. A thorough knowledge on judicious oral hygiene practices, including the use of non-abrasive toothpastes and proper brushing techniques can reduce the intensity of mechanical wear. Modification of dietary habits through counselling may be effective to minimise acidic food intake and additionally, addressing any underlying systemic conditions such as gastroesophageal reflux disease (GERD) can help prevent erosion[11]. Any further deterioration of tooth structure, due to severe wear can be prevented through restorative procedures including composite resin buildup, that tend to provide additional protective barriers against the same.

To date several therapeutic strategies have been tried for combating cases of severely worn-out dentition. Of all, the most comprehensive modality, which involves a multidisciplinary approach is the full mouth rehabilitation (FMR)[6,12].

The restorative techniques encompasses direct and indirect restorations with composite restorations, porcelain veneers, and even full coverage crowns. The prosthetic rehabilitation system comprises of the removable or fixed prostheses to restore normal occlusion with thorough occlusal adjustments for implementing the most ideal occlusal relationship so as to ensure utmost comfort and long-term stability for the patient. Several FMR philosophies are in concurrent practice, all of which aim to achieve one goal, which is the harmonious occlusal contacts, that will eventually maintain TMJ in health and prevent further tooth structure loss[13]. It is also mainly designed to re-establish the VDO which ensures the optimised adaptation in patients before the final prosthetic placements.

The present case report describes the full-mouth rehabilitation of a 58-year-old male patient diagnosed with Turner and Missirlian Category I wear[2,14]. The treatment plan involved a multidisciplinary treatment approach with periodontal plastic surgeries, fixed and removable prostheses to restore function, aesthetics, and occlusal stability.

Case Description

History

A 58-year-old male patient, presented with the primary complaint of missing teeth in the upper and lower right and left posterior regions for the past two years and severe attrition of the remaining dentition. He also reported difficulty in mastication with a gradual decline in his ability to chew solid foods. The patient was also concerned about the aesthetic deterioration of his teeth and the deranged facial appearance due to worn-out dentition. On eliciting the history, the patient reported a long-standing history of progressive tooth wear, which was first noticed approximately ten to twelve years ago. At first, it was non-attended as the condition did not interfere with his routine activities or his social well-being. However, the problem had gradually worsened over the past five years, accompanied by tooth hypersensitivity and difficulty in chewing. History also highlighted the fact, that he does not exhibit any parafunctional habits such as bruxism or nail-biting but admitted to consuming acidic foods and beverages more frequently. The patient also presented with non-contributory medical history, affecting oral health.

Clinical examination and diagnosis

The extraoral examination revealed a symmetrical facial structure with an ovoid facial form and a straight profile. There were no signs of lymph node enlargement, cutaneous ulcers, scars, or any exophytic growths. A thorough TMJ assessment showed no signs of pain, clicking sounds, or crepitus. Additionally, there was no observed dysfunction related to the TMJ or the muscles of mastication. The mandible exhibited no deviation, indicating normal function and alignment **(Figure 1A).**

A thorough intra-oral examination was conducted and was identified that the gingival health was generally satisfactory, with no clinical signs of inflammation. No signs of soft tissue pathology or other abnormalities were detected. On examining the hard tissues, there were multiple missing teeth. Generalized attrition was noted across all remaining teeth, especially in the anterior and posterior occlusal surfaces. The attrited teeth presented with sharp enamel edges and dentinal craters. There was no active dental caries, however, the remaining teeth had undergone root canal treatment, including 11, 12, 13, 17, 21, 22, 23, 27, 31, 32, 33, 34, 35, 41, 42, 43, 44, 45, and 47. The clinical findings were confirmed with radiographic examination using orthopantomogram (OPG) **(Figure 1B-F).** Centric relation-centric occlusion discrepancy was observed when the patient was guided into the centric relation position using Dawson bimanual method. Extreme care was taken to determine for alterations in the VDO which consisted of the following steps. Firstly, the history of wear was identified and was found that the patient exhibited the habit of consuming more of acidic foods and beverages. Secondly, the interocclusal space was determined to be more than 4mm. Thus, the possible causes of wear was established to be majorly due to the frequent consumption of acidic foods, and secondly, due to the posterior interferences. The patient was diagnosed with Turner and Missirlian Category I tooth wear due to excessive wear with loss of VDO[14]. The diagnosis was confirmed based on clinical findings of generalized attrition and loss of posterior support, decreased lower facial height, functional impairment, and radiographic evidence of bone loss and extensive previous restorations. However, differential diagnoses was made to rule out other potential possibilities including GERD- associated erosive tooth wear, bruxism-related attrition, and severe abrasion due to improper tooth brushing habits. No evidence of parafunctional habits was found, and the wear pattern was consistent with occlusal overload.



Figure 1. Clinical photographs of extra-oral and intra-oral examination



Figure 2. Orthopantomogram with attrition of the remaining dentition and evidence of RCT

Comprehensive Treatment Plan and Clinical Procedure for Full-Mouth Rehabilitation

A comprehensive treatment plan was formulated to ensure long-term stability, function, and aesthetics. Several treatment options were considered, including removable partial denture, overlay denture, and full-mouth composite resin build-up. However, these were ruled out in favor of a fixed rehabilitation plan that offered long-term stability, and predictability.

The selected approach involved post and core restorations to reinforce structurally compromised teeth and provide additional retentive support. Full-mouth surgical crown lengthening was performed to increase the crown height, ensuring optimal retention for full-coverage crowns and facilitate proper occlusal function and aesthetics. A combination of fixed and removable prostheses was planned with porcelain fused to metal crowns (PFM) supported fixed partial dentures for root canal-treated teeth and removable denture for the posterior edentulous areas. Implants were also recommended for the patient, but they could not be implicated in the treatment due to economic constraints of the patient.

After thoroughly explaining the treatment plan to the patient and obtaining written informed consent, the rehabilitation process was initiated. The initial step involved complete oral prophylaxis, followed by post and core restorations for additional reinforcement **(Figure 3A-C).** Surgical crown lengthening procedure was carried out in the mandibular arch which displayed an uneventful post-operative healing. **Figure 3D-I** shows the intra-operative procedure and its healing at 1 week follow-up. This ensured an increase in the crown height of approximately 2 mm which was sufficient for adequate retention of full coverage ceramic crowns. **Figure 4** shows the OPG after the placement of cast posts. This was followed by the re-establishment of appropriate VDO using the wax occlusal rims with a rise of 4mm **(Figure 5A)**, in-turn, followed by fabrication of fixed partial denture with PFM crowns placed in the maxillary region (teeth 13-23) and the mandibular region (teeth 35-47) **(Figure 5B-F).** Additionally, Co-Cr crowns were placed on teeth 18 and 27 with mesial occlusal rests over which the cast partial denture was supported. In order to restore the posterior edentulous sites, attachment-retained cast partial denture was fabricated for teeth 14-17 and 24-26 in the maxillary arch supported on the mesial occlusal rests on 18 and 27 respectively and precision attachment with respect to the corresponding premolars. Finally, a precision attachment denture was provided for teeth 36 and 37 in the mandibular arch **(Figure 6A-F).** Patient's adaptation to the increased VDO was monitored using provisional restorations for a period of three months and the absence of muscle tenderness, functional discomfort and temporomandibular joint discomfort ensured the patient's adaptation to the re-established VDO, before the placement of the final prosthesis. The patient was kept under regular maintenance protocol and four reviews were completed in a period of about one year **(Figure 7).**



Figure 3. A and B. Shows post space preparation; C. Cast posts cemented to their respective teeth; D-F, Surgical crown lengthening procedure in the mandibular arch; G-I, 1 week postoperative healing

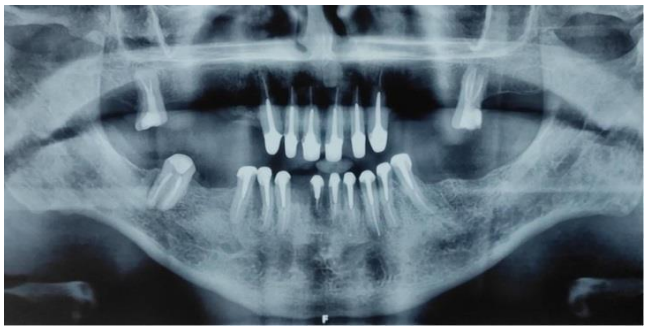


Figure 4. OPG showing cast posts placement



Figure 5. Wax occlusal rims showing increase in VDO of 4 mm; B and C, refinement of the cast posts; D-F, metal try-in



Figure 6. A, Final prosthesis; B-F, Post-operative view after full mouth rehabilitation



Figure 7. One year post-operative OPG showing successfully restored oral function through systematic FMR

DISSCUSION

Management of a case with severely worn out dentition along with loss of vertical dimension, presents numerous challenges to the clinician[15]. The primary objective of the current treatment plan was to restore function and aesthetics, without compromising the occlusal harmony, also while assuring long-term stability and durability of the intended treatment strategy. The present case encompassed a multidisciplinary approach, including endodontic therapy, periodontal therapy, and prosthetic replacements, which comprehensively strived to accomplish a successful full mouth rehabilitation. This approach was in accordance with previous literature of successful and stable rehabilitation[1,9].

The most important challenge in the current case was evaluation of the VDO, which was systematically analysed and gradually implemented. The use of provisional prosthesis for monitoring the patient's adaptation for about three months was the key in attaining the most possible and stable functionality. This was in accordance with the interim time period as reported by a previous study[1]. Evidence suggest that, VDO, which is arbitrarily estimated without a systematic evaluation, could eventually result in multiple complications. In addition, the interim period for patients adaptability could be modified according to the patient's situation[1]. A previous study has also suggested that controlled increase in VDO could result in positive neuromuscular adaptation when done gradually[9].

Another commentative challenge in this case was the management of short clinical crowns, which could severely affect the retention of restorations, if not cared for. To address this, surgical crown lengthening was performed to provide sufficient coronal structure for retention of prosthetic restorations. Previous reports from literature have immensely supported crown lengthening to be the most effective way to enhance retention and thus, longevity of full-coverage restoration[16–18]. Seol *et al.*, in their study have also demonstrated the effectiveness of combining surgical crown lengthening with full-mouth rehabilitation to restore occlusal function and aesthetics[17]. Kumar *et al.*, and Gupta *et al.*, have previously highlighted the role of cast post-and-core restorations in cases where structural integrity is compromised[19,20]. The present case adopted a similar approach by using cast post-and-core to reinforce weakened teeth before final crown placement.

Additionally, occlusal stability was ensured by implementing a mutually protected occlusal scheme, which has been recommended in the literature as an optimal approach for rehabilitating severely worn dentition[9]. The final restorations were fabricated using porcelain-fused-to-metal crowns, balancing durability and aesthetics. Previous study has also reported that PFM crowns serve as the most viable option for restoring the form and function of teeth while enhancing esthetics and comfort [21]. Moreover, removable cast partial denture was an absolute indication in the present case which in turn, exhibited a positive impact on the successful outcome of rehabilitation. Such removable prostheses are frequently recommended in cases where, fixed options are contraindicated or when they are expected to yield suboptimal clinical results[15].

A key aspect of this case was the gradual increase in VDO using provisional restorations, which allowed neuromuscular adaptation and prevented complications. Additionally, surgical crown lengthening provided adequate retention for full-coverage crowns, improving prosthetic longevity. The integration of fixed and removable prostheses addressed occlusal stability and patient-specific needs. By following a structured protocol and leveraging evidence-based techniques, a predictable and durable outcome was achieved.

CONCLUSION

The successful management of severely worn-out dentition requires a structured, multidisciplinary, and evidence-based treatment approach. This case emphasized the importance of a comprehensive assessment, including VDO evaluation, occlusal analysis, and patient adaptation monitoring. The combination of endodontic, periodontal, and prosthetic interventions ensured functional rehabilitation, long-term stability, and aesthetic enhancement. This case underscores the significance of individualized treatment planning, meticulous execution, and long-term follow-up in restoring function and aesthetics in patients with severe tooth wear.

Declarations

Acknowledgment

Nil

Conflict of interest

The authors declare no conflict of interest

Financial disclosures

Nil

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CASE REPORT

CONGENITAL NASAL GROWTH IN A NEWBORN:A CASE REPORT ON DIAGNOSIS AND SURGICAL MANAGEMENT

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ABSTRACT

Background: Congenital nasal masses, although rare, pose significant clinical challenges, particularly in neonates. These masses may be benign or malignant, and careful monitoring and early intervention are essential to avoid complications. This case report describes a male neonate with a congenital growth at the tip of his nose.

Material and Methods: We report the case of a 1,5-year-old child who were treated for nasal growth in our unit, inclusive, were reviewed retrospectively. Information recorded included medical history, clinical characteristics, diagnostic procedures, treatment methods, and the outcome.

R**esults**: At six months mass was removed under general anesthesia without complication. This case highlights the importance of early diagnosis and timely surgical treatment for congenital nasal masses, ensuring both functional and cosmetic benefits.

Conclusion: Congenital nasal masses, though rare, require careful monitoring and timely intervention to prevent complications

Keywords: newborn, congenital anomalies of the nose, congenital nasal masses, teratoma**.**

INTRODUCTION

Congenital nasal masses are a consequence of disrupted embryonal developmental processes in the frontonasal region. They are thought to be embryologically related to developmental anomalies of the frontal lobe and the responsible mechanism is the incorrect closure of the anterior neuropore, which leads to the preservation of the anterior cranial defect[1,2] Embryonic abnormalities in the frontal lobe can result in the development of midline nasal masses[3].

Congenital nasal masses are rare and can be composed of tissue that has herniated from the cranium or may be made of tissue native to the nasal cavity. Encephaloceles, Meningocele and Gliomas can consist of ectopic intracranial tissue and may have an intracranial connection[4-6]. Dermoids in contrast are not composed of intracranial tissue and are thought to be due to the failure of the normal separation of the different germ cell layers. There are a wide range of causes of nasal masses in children[6-9].

Congenital nasal masses, though infrequent, are critical to recognize due to their potential for functional and cosmetic complications.

Early diagnosis and intervention are essential to avoid complications such as airway obstruction or infections[11-13].

Diagnosis of congenital anomalies of the nose is based on collecting anamnesis, objective examination of the child, results of laboratory tests and instrumental diagnostic methods. Anamnestic data can indicate a possible etiology - previous infections, exposure to pathological factors, genetic predisposition, etc. Severe defects of the external nose are manifested by characteristic deformation of the facial skull already during the initial examination by a pediatrician or neonatologist. In the development of sinusitis, this technique reveals pallor, excessive moisture of the mucous membranes. Laboratory tests are used to confirm or refute infectious etiology, exclude inflammatory complications. Instrumental research methods occupy a leading place in the diagnosis of congenital anomalies of the nose. In pediatrics, nasal cavity probing, fibroendoscopy, radiography (sometimes with contrast). Probing makes it possible to determine the patency of the nasal passages, fibroendoscopy – to fully examine the condition of all intranasal structures[14-16]. Radiographic work-up and management of any neonatal respiratory distress that may result from absent or hypoplastic nasal structures should be performed expeditiously. Computed tomography (CT) or magnetic resonance imaging (MRI) remain the standardsfor presurgical management in all midface nasal masses. A CT image is preferred for assessing the bony defect. Nasal gliomas may contain areas of calcification or cystic changes on CT scan and tend to be isodense on CT.

Magnetic resonance imaging (MRI) provides detailed imaging of the nose and post nasal space and has the advantage of soft tissue imaging and information on any intracranial connection. An MRI can differentiate between gliomas and encephalocele by demonstrating an associated CSF space.

Surgery is the only treatment modality to prevent local and intracranial inflammatory complications, as well as distant facial skeletal deformities[17-19].

In newborns there may be a visible mass projecting from the nasal cavity and may present with respiratory distress due to the fact that newborns tend to be obligate nose breathers. They may become cyanotic when feeding or crying, a condition known as cyclic cyanosis. The timing of surgical intervention in patients with congenital nasal anomalies depends on the specific pathology. Newborns are required to breathe through their nose for at least 6 weeks of life and up to the first 6 months.

The main treatment for congenital nasal anomalies is surgical. The time frame for the operation depends on the severity of the child's condition. In case of choanal atresia, the earliest possible correction is indicated, since there is a high risk of asphyxia. Severe deformations of the external nose are also subject to rhinoplasty at an early stage. The scope of operations and surgical access are determined individually for each child. External lesions may be excised using a skin incision over the mass or a coronal flap approach. After the treatment, there is always a rehabilitation period, including full care, regular treatment of the mucous membranes of the nasal cavity, and drainage changes every 10-15 days. If necessary, the child is transferred to parenteral nutrition; infusion and antibacterial therapy can be used.

This case report discusses a neonate with a congenital nasal growth that was diagnosed and treated surgically, providing an opportunity to highlight the clinical and diagnostic steps involved in managing such conditions.

Case Report

The case that we present is unique in the Republic of Armenia, congenital growth of the nose in a child. A

male infant, born on December 28, 2023, was diagnosed with a congenital growth at the tip of his nose (Figure 1A, B).

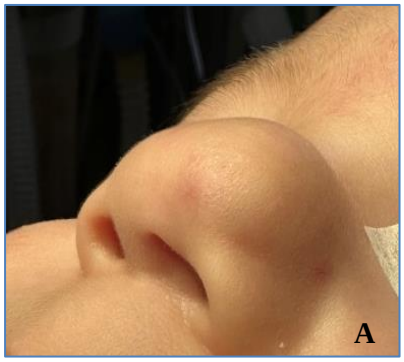




Figure 1. A,B Growth at the tip of nose.

The lesion was immediately observed following birth, and the pediatric team initiated periodic monitoring. By three months, the lesion showed noticeable growth, prompting further investigation. Sonographic assessments revealed an increase in size, and an MRI was ordered at that time.

The MRI confirmed the presence of a benign soft tissue mass, likely a dermoid cyst, and it indicated the necessity for surgical intervention(Figure 2).



Figure 2. The MRI of the growth.

At six months of age, the infant was admitted to the maxillofacial surgery department at Yerevan State Medical University, Muratsan Hospital Complex. The mass was excised under general anesthesia without complications.



Figure 3. Immediately after removing growth mass

The excised tissue was sent for histopathological examination(fig.4), which confirmed the diagnosis of a **teratoma**. The biopsy revealed a well-circumscribed lesion composed of stratified squamous epithelium with a fibrous capsule and sebaceous glands. There were no signs of malignancy or infection, and the cystic contents were found to contain keratinized material. These histological features are typical of dermoid cysts, confirming the benign nature of the mass.



Figure 4. Excised tissue which undergo histopathological examination

The infant's recovery following surgery was uneventful. No complications such as infection, wound dehiscence, or cosmetic concerns were noted. The postoperative outcome was excellent, with no recurrence of the lesion after several months of follow-up.

DISCUSSION

Congenital nasal masses are rare but important to diagnose early, as they can lead to significant functional and aesthetic issues[20,21].

Masses in the nose may be caused by glioma, meningoencephalocele, encephalocele, congenital rhabdomyosarcoma, and lymphatic malformations[22].

Teratomas are thought to arise from multipotent germ cells anywhere from the brain to the coccyx, most often in the midline during the fourth and fifth weeks of gestation[22]. The estimated reported incidence of teratomas is 1 in 35,000–40,000 live births[23,24].

Most teratomas arising in the head and neck region are benign and asymptomatic, can cause upper airway obstruction and respiratory distress in newborns, and require intubation to maintain airway patency and ensure early removal[25,26].

Imaging plays a crucial role in the evaluation of congenital nasal masses. Ultrasound is often used as the first-line imaging technique for soft tissue evaluation in neonates. However, when further detail is needed, MRI is the preferred modality. Surgical excision remains the treatment of choice for symptomatic congenital nasal masses, especially those that are progressively growing or causing cosmetic concerns. Early surgical intervention, as in this case, typically leads to a favorable outcome both functionally and cosmetically.

The prognosis for life in children with congenital nasal anomalies, provided that the operation is performed in a timely manner, is favorable. The cosmetic result directly depends on the severity of the defect and the capabilities of the pediatric surgeon. In most patients, it is possible to reconstruct the physiological shape of the nose, restore normal nasal breathing and patency of the paranasal sinuses. Prevention of congenital nasal anomalies is similar to any other congenital pathologies. It involves adequate pregnancy management, regular visits to the antenatal clinic, ultrasound according to the schedule, and complete rejection of bad habits during pregnancy. This case illustrates the importance of early intervention and the successful outcome that can result from appropriate management of congenital nasal growths.

CONCLUSION

Congenital nasal masses, though rare, require careful monitoring and timely intervention to prevent complications. Early diagnosis through imaging, including sonography and MRI, is crucial to determining the appropriate management plan. Surgical excision is often the treatment of choice for lesions that are causing symptoms or growing progressively.

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The authors declare no conflict of interest

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Ethical approval

In this study ethical approval is not required.

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REVIEW ARTICLE

FROM ORAL CARE TO VIRUS CONTROL: BIOMEDICAL APPLICATIONS OF CETYLPYRIDINIUM CHLORIDE – A SYSTEMATIC REVIEW

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ABSTRACT

Background: Cetylpyridinium chloride (CPC) is a quaternary ammonium compound extensively used in oral hygiene products for its potent antimicrobial and antiviral activity. Its mechanism involves disruption of microbial cell membranes through electrostatic and hydrophobic interactions. In addition to its oral applications, CPC has gained attention for integration into biomaterials and potential to mitigate SARS-CoV-2 transmission, warranting a comprehensive review of its biomedical relevance.

Materials and Methods:A systematic review was conducted following PRISMA guidelines across PubMed, ScienceDirect, and Google Scholar (1990–2023), using terms related to CPC's antimicrobial, antiviral, and material-incorporation functions. Eligible studies included in vitro, in vivo, and clinical investigations assessing CPC's efficacy, mechanisms, and integration into dental materials. Articles were screened, data extracted, and quality assessed using CONSORT and STROBE tools. Due to heterogeneity, findings were synthesized qualitatively.

Results: Evidence supports CPC's significant antimicrobial efficacy in reducing plaque, gingivitis, and biofilm formation, with up to 30% improvement over controls. It exhibits broad-spectrum bactericidal action, though polymicrobial biofilms show reduced susceptibility. CPC also demonstrates >99.9% inactivation of SARS-CoV-2 in vitro, indicating prophylactic potential. Its incorporation into orthodontic adhesives and resins yields sustained antimicrobial effects, though material strength may decline over time. Emerging resistance and biofilm penetration barriers highlight key limitations.

Conclusion:CPC is a versatile antimicrobial with proven utility in oral care and public health. Its efficacy against bacterial and viral pathogens, including SARS-CoV-2, and potential for biomaterial integration underscore its translational value. However, long-term biocompatibility and resistance development require further research to optimize its application across clinical domains.

Keywords: Cetylpyridinium Chloride (CPC); Antimicrobial Activity; Oral Biofilms; SARS-CoV-2 Inactivation; Biomedical Applications

INTRODUCTION

Cetylpyridinium chloride (CPC) is a quaternary ammonium compound that functions as a cationic surfactant with strong amphiphilic properties. These characteristics allow CPC to interact effectively with microbial cell membranes, disrupting their structural integrity and leading to cell lysis and death. Its mode of action involves the electrostatic attraction between the positively charged pyridinium ion and the negatively charged components of bacterial membranes such as lipopolysaccharides and phospholipids. Additionally, the hydrophobic cetyl chain intercalates into lipid bilayers, causing membrane disorganization, leakage of cytoplasmic contents, and ultimately, microbial death.[1-3]

These properties confer CPC broad-spectrum antimicrobial activity against both gram-positive and gram-negative bacteria, as well as certain fungi and enveloped viruses.[4]

CPC is widely used in a range of oral hygiene products, including toothpastes, mouthwashes, lozenges, and sprays due to its efficacy in reducing dental plaque, gingivitis, halitosis, and bacterial load in the oral cavity.[5-7] Its effectiveness in disrupting oral biofilms has made it a preferred adjunct in both preventive and therapeutic dentistry.[8] Beyond dentistry, CPC has garnered attention for its antiviral activity, particularly against SARS-CoV-2, where studies demonstrated its ability to reduce viral load in saliva by up to 99.9% following short-term exposure[9], making it a valuable candidate for reducing viral transmission risk. Furthermore, emerging research suggests that CPC can be integrated into biomedical materials, such as orthodontic adhesives and denture bases, to provide sustained antimicrobial effects.[10] However, issues such as microbial resistance, material compatibility, and formulation challenges continue to be explored. This systematic review comprehensively analyzes the literature on CPC's biomedical applications, including its antimicrobial efficacy, antiviral capabilities, material incorporation, resistance development, and safety profile. The goal is to provide a well-rounded understanding of CPC's therapeutic value and future potential in healthcare and public health settings.

2. MATERIAL AND METHODS

2.1 Search Strategy and Data Sources

A structured and comprehensive systematic review was performed in accordance with PRISMA guidelines to collate existing literature on the biomedical applications of Cetylpyridinium chloride (CPC). An exhaustive electronic search was conducted across three major scientific databases: PubMed, Google Scholar, and ScienceDirect, covering a publication period from January 1990 to December 2023. The search strategy incorporated Boolean logic and truncation operators using the following terms: "*Cetylpyridinium chloride" OR "CPC" AND "oral biofilm" OR "antimicrobial mouthwash" OR "SARS-CoV-2 CPC" OR "CPC resistance" OR "orthodontic adhesives" OR "CPC dental materials"*. Additionally, manual reference mining of retrieved articles and reviews was performed to ensure inclusion of all pertinent studies.

2.2 Eligibility Criteria

Studies were selected based on pre-defined inclusion and exclusion criteria. Eligible studies included:

- Original experimental research involving in vitro, in vivo, or ex vivo models;
- Clinical investigations such as randomized controlled trials (RCTs), cohort studies, or case-control studies;
- Studies reporting on CPC efficacy, mechanism of action, resistance development, antiviral applications, or material incorporation.

Only articles published in peer-reviewed journals and written in English were considered. Exclusion criteria comprised commentaries, editorials, conference abstracts, duplicate reports, and review articles that did not provide new experimental data or systematic synthesis.

2.3 Study Selection and Data Extraction

All articles were independently screened in two stages by two reviewers (blinded to author identities). First, titles and abstracts were reviewed for relevance. Full texts of potentially eligible studies were then assessed in detail. Discrepancies were resolved through discussion or consultation with a third reviewer. The PRISMA flowchart guided the selection process as illustrated in Figure 1. After removing duplicates, titles and abstracts were screened for relevance. Full-text reviews of eligible studies followed, ensuring compliance with the inclusion criteria. Data were extracted using a standardized data extraction form designed to capture study characteristics, including:

- Authors, publication year, and journal;
- Study design and experimental setting;
- CPC concentration and formulation;
- Microbial or viral targets;
- Quantitative and qualitative outcomes;
- Findings, limitations, and conclusions.

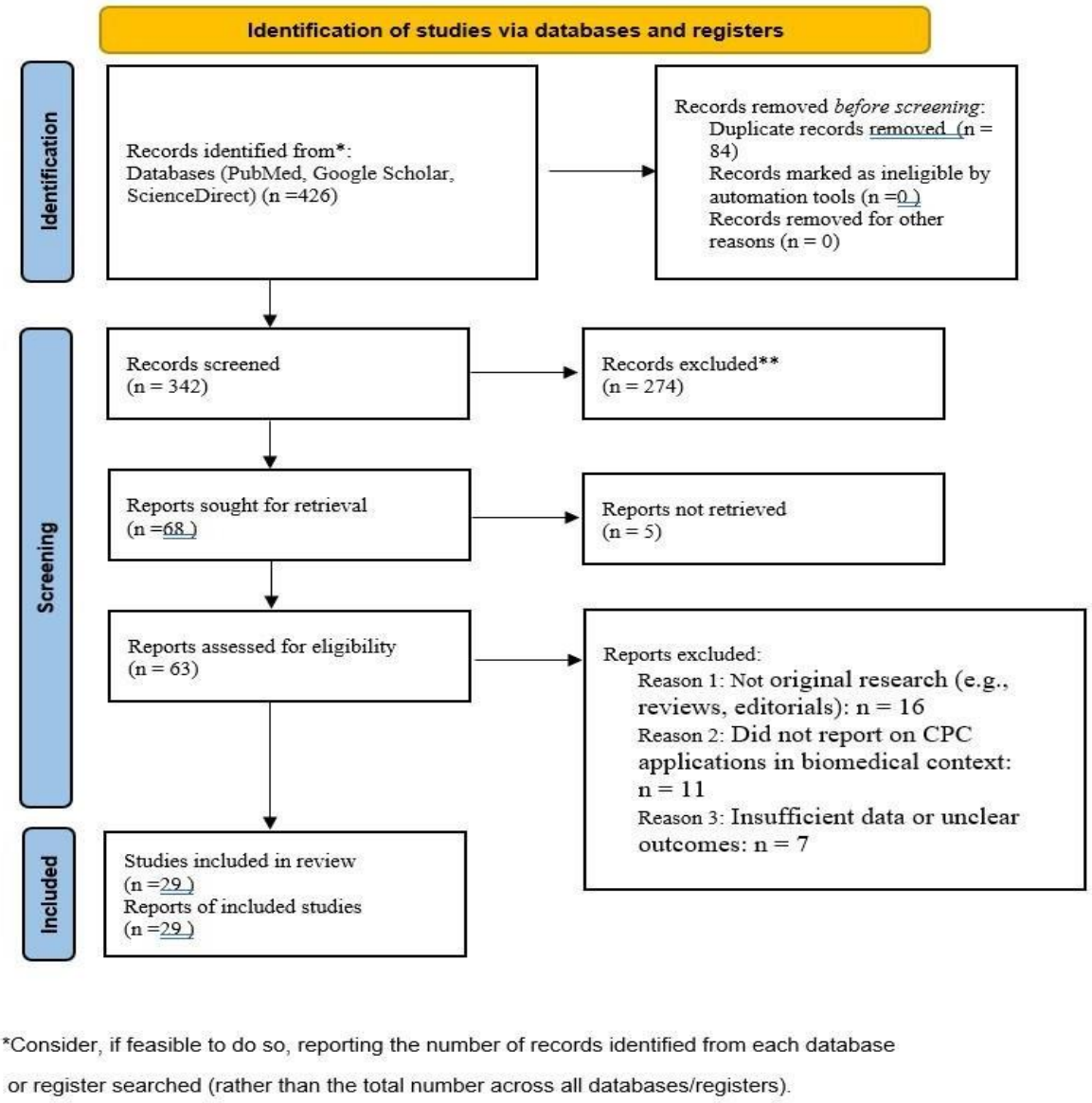


Figure 1. PRISMA flowchart

2.4 Quality and Risk of Bias Assessment

To evaluate the internal validity of included studies, two reviewers independently assessed the risk of bias using established tools:

- CONSORT 2010 checklist for clinical trials;
- STROBE guidelines for observational and laboratory-based studies.

Assessment criteria included randomization, blinding, sample size justification, statistical rigor, reproducibility, and transparency in outcome reporting. Any discrepancies in assessment were resolved through consensus or adjudication by a senior investigator.

2.5 Data Synthesis

Due to the heterogeneity of study designs, interventions, and outcomes, a qualitative synthesis was performed. Results were stratified by application domains (e.g., antimicrobial efficacy, antiviral activity, biomaterial integration, resistance mechanisms) and CPC concentrations. Where applicable, outcome trends were highlighted and compared across studies.

3. RESULT

Table 1. Summary of Key Findings on the Biomedical Applications of Cetylpyridinium Chloride (CPC)

Application Domain	Study/Authors	Model/Setting	CPC Formulation & Dose	Target Organism(s)	Key Findings	Limitations
Antimicrobial (Oral)	Stookey et al. (2005) [5]	RCT (6 months)	0.075% & 0.10% mouthwash	Oral plaque biofilms	~30% reduction in plaque and gingival inflammation	Limited to short-term safety
	He et al. (2012) [6]	Clinical trial	0.07% CPC rinse	Supragingival plaque bacteria	Sustained antimicrobial activity up to 12 hrs	No long-term resistance tracking
	Lotufo et al. (2009) [7]	Clinical study	0.05% CPC rinse	Oral biofilm vs fluoride rinse	29.3% superior plaque reduction vs fluoride	Small sample size
	Rao et al. (2011) [8]	In vitro multi-species biofilm	Alcohol-free CPC rinse	Mixed oral flora	Disruption of oral biofilms	Not validated in vivo
Mechanism of Action	Gilbert & Moore (2005) [2]	Theoretical review	N/A	Gram-positive/negative bacteria	Displacement of Ca^{2+}/Mg^{2+}, membrane disruption, cell lysis	No clinical testing
	Denyer & Stewart (1998) [3]	Microscopy & enzymatic assays	N/A	Bacterial membranes	Induces autolysis, metabolic inhibition	Primarily mechanistic
Resistance Development	Luppens et al. (2008) [11]	In vitro biofilms	0.07% CPC	S. mutans + V. parvula	Dual-species biofilms more tolerant than mono	Specific to species studied
	Smith et al. (2013) [18]	In vitro	Commercial rinses with CPC	MRSA	<60% biofilm reduction	Not effective on resistant strains
	Tattasawart et al. (1999) [17]	Adaptive exposure	Repetitive sub-lethal doses	P. stutzeri	60× MIC increase over 6 weeks	High resistance potential
	Izano et al. (2008) [12]	Enzymatic biofilm disruption	CPC + Dispersin B	A. actinomycetemcomitans	EPS degradation restored CPC efficacy	Only tested one EPS enzyme
Antiviral (SARS-CoV-2)	Anderson (2021) [9]	In vitro plaque assay	CPC mouthrinse (0.075%)	SARS-CoV-2 variants	>99.9% reduction in viral titers after 30 sec	Not tested in vivo

Application Domain	Study/Authors	Model/Setting	CPC Formulation & Dose	Target Organism(s)	Key Findings	Limitations
	Perez-Errazuriz et al. (2021) [14]	In vitro	0.07% CPC rinse	SARS-CoV-2 (P.1, B.1.1.7)	Broad virucidal activity across strains	Pre-clinical only
Material Integration	Al-Musallam et al. (2006) [10]	In vitro resin testing	CPC at 2.5%, 5%, 10% in adhesives	S. mutans	Dose-dependent inhibition; antibacterial resins	DTS reduction over time
	Ganeshnarayan et al. (2009) [13]	Diffusion modeling	CPC transport studies	Poly-N-acetylglucosamine biofilms	EPS blocks CPC diffusion	Limited to laboratory conditions

3.1 Antimicrobial Efficacy of CPC in Oral Applications

A substantial body of clinical and in vitro research confirms the potent antimicrobial activity of Cetylpyridinium chloride (CPC) against diverse oral pathogens. In a large-scale randomized controlled trial, **Stookey et al. (2005)** evaluated two concentrations of CPC mouthwashes—0.075% and 0.10%—over a six-month period and reported statistically significant reductions in plaque accumulation, gingival inflammation, and bleeding indices, with reductions of up to 30% compared to placebo. These findings were corroborated by **He et al. (2012)** and **Rao et al. (2011)**, who demonstrated that both single-use and prolonged application of CPC-containing rinses resulted in substantial suppression of microbial biofilms in the supra-gingival plaque region.

Furthermore, **Lotufo et al. (2009)** found that a 0.05% CPC rinse reduced plaque accumulation by 29.3% more than a fluoride-based control rinse 12 hours post-application. These results confirm that CPC has a broad-spectrum antimicrobial profile, effectively targeting both gram-positive and gram-negative bacterial species implicated in caries, periodontitis, and halitosis. CPC's substantivity—its ability to remain adsorbed on oral surfaces and provide prolonged antimicrobial effects—further contributes to its efficacy.

3.2 Mechanism of Action

The bactericidal action of CPC is primarily attributed to its cationic pyridinium moiety, which disrupts ionic homeostasis by displacing essential divalent cations such as calcium and magnesium from microbial cell surfaces (Gilbert & Moore, 2005). This displacement compromises cell wall integrity, facilitating the insertion of CPC's hydrophobic cetyl chain into the lipid bilayer, which destabilizes membrane structure and induces cell lysis.

Moreover, CPC initiates autolytic enzymatic activity, triggering the degradation of structural proteins and nucleic acids within microbial cells. **Denyer and Stewart (1998)** report that CPC interferes with intracellular metabolic processes, including RNA synthesis and protein folding, contributing to irreversible cell death. Scanning electron microscopy and fluorescence assays have shown clear evidence of membrane disruption and cytoplasmic leakage following CPC treatment.

3.3 Resistance Concerns and Limitations

While CPC is generally effective, emerging studies suggest that biofilm-associated bacteria may exhibit reduced sensitivity to CPC. **Luppens et al. (2008)** demonstrated that dual-species biofilms composed of *Streptococcus mutans* and *Veillonella parvula* exhibited significantly higher tolerance to CPC than single-species biofilms. This suggests synergistic protective mechanisms within polymicrobial communities that hinder CPC penetration or inactivate its surfactant properties.

Likewise, **Smith et al. (2013)** evaluated CPC efficacy against methicillin-resistant *Staphylococcus aureus* (MRSA) and reported less than 60% reduction in biofilm viability, suggesting limited effectiveness against certain antibiotic-resistant strains. Furthermore, **Tattasawart et al. (1999)** observed an increase in CPC minimum inhibitory concentrations (MICs) by up to

60-fold in *Pseudomonas stutzeri* after six weeks of repeated sub-lethal exposure, indicating the potential for adaptive resistance.

The extracellular matrix components such as poly-N-acetylglucosamine (PNAG) have also been implicated in CPC resistance. **Izano et al. (2008)** demonstrated that enzymatic degradation of PNAG using dispersin B significantly increased CPC susceptibility in *Aggregatibacter actinomycetemcomitans*, highlighting the barrier role of exopolysaccharides.

3.4 Antiviral Activity Against SARS-CoV-2

Recent investigations have extended the applicability of CPC into virology. In the context of the COVID-19 pandemic, CPC has demonstrated virucidal activity against multiple SARS-CoV-2 variants. **Anderson (2021)** and **Perez-Errazuriz et al. (2021)** reported that CPC-containing mouthrinses achieved a >99.9% reduction in viral titers after only 30 seconds of exposure in vitro plaque assays. These results were consistent across multiple viral lineages, including B.1.1.7 and P.1.

The mechanism involves CPC's ability to disrupt the viral lipid envelope, rendering the virus non-infectious. These findings suggest that routine oral hygiene practices using CPC mouthrinses could serve as a non-invasive strategy to reduce viral load in saliva, potentially limiting transmission in clinical and community settings. Given the high viral load in the oropharynx during early infection, CPC rinses may offer prophylactic benefit to both infected individuals and close contacts.

3.5 Integration into Dental Materials

Beyond topical formulations, CPC has been explored for incorporation into biomaterials used in dental and orthodontic applications. **Al-Musallam et al. (2006)** investigated the antimicrobial efficacy of orthodontic adhesives modified with CPC concentrations of 2.5%, 5%, and 10%. The study found a dose-dependent reduction in bacterial colonization, particularly against *S. mutans*, a key etiological agent in enamel demineralization.

However, material characterization revealed a reduction in diametral tensile strength (DTS) following water aging, particularly at higher CPC concentrations. While polymerization and setting of the material remained unaffected, the mechanical compromise raises questions about long-term durability and biocompatibility. Despite these concerns, the ability to deliver sustained antimicrobial activity makes CPC-modified resins a promising innovation for denture bases, retainers, and intraoral devices, especially in high-risk patients.

DISCUSSION

The findings of this systematic review reinforce the broad-spectrum antimicrobial and antiviral efficacy of Cetylpyridinium chloride (CPC), particularly in applications related to oral health and hygiene. CPC demonstrates potent activity against a range of pathogenic microorganisms, including *Streptococcus mutans*, *Aggregatibacter actinomycetemcomitans*, and other contributors to caries and periodontal diseases.[5,6,10] In monomicrobial environments and controlled in vitro settings, CPC's mechanism of action—disruption of the cell membrane via cationic interaction and lipid bilayer insertion—yields consistently favorable outcomes in reducing biofilm mass, plaque indices, and microbial load.[1-3]

However, microbial biofilms present a formidable barrier to CPC penetration. The extracellular polymeric substances (EPS) within biofilms create a physical and chemical diffusion barrier that reduces CPC's access to inner microbial layers. Studies involving dual-species and polymicrobial biofilms have reported diminished efficacy, as evidenced by *Streptococcus mutans* demonstrating increased tolerance when co-cultured with *Veillonella parvula*.[11] These observations highlight the importance of exploring synergistic combinations, such as CPC with enzymatic agents like dispersin B (DspB), which degrades EPS components and facilitates deeper penetration and antimicrobial action.[12,13] Such combinations may enhance CPC's biofilm-disruptive potential and address limitations in resistant microbial communities.

Beyond its oral health applications, CPC has gained relevance in broader public health contexts, especially during the COVID-19 pandemic. Its demonstrated virucidal activity against SARS-CoV-2 and other enveloped viruses supports its inclusion in antiviral mouthrinses and preventive protocols.[9,14] In addition, CPC's utility has been extended to buccal tablets, hand sanitizers, disinfectant sprays, and edible surface sanitizers. Regulatory bodies such as the FDA and EFSA have approved CPC for use on produce and poultry surfaces, citing its low toxicity and broad antimicrobial spectrum.[15,16]

Despite these promising attributes, long-term safety and biocompatibility concerns must be addressed.

Studies on CPC-integrated dental biomaterials, such as orthodontic adhesives and acrylic resins, report mechanical degradation after aging, raising questions about material stability and suitability for prolonged intraoral use.[10] Furthermore, the potential for resistance development, particularly in hospital and community environments where CPC is frequently used, necessitates ongoing surveillance, resistance profiling, and the development of alternating formulations or rotational use protocols.[11,17-19]

In conclusion, while CPC holds significant promise as a multifunctional antimicrobial agent, its optimal use requires continued innovation in formulation science, strategic combination therapies, and robust clinical validation in long-term settings.

5. CONCLUSION

Cetylpyridinium chloride remains a valuable antimicrobial and antiviral agent, with significant contributions to oral hygiene, disinfection, and infection control. Its future lies in enhanced delivery mechanisms and integration into smart dental materials. Resistance surveillance and formulation stability are crucial in maximizing its therapeutic benefit.

6. DECLARATIONS

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Conflict of interest

All the authors declare that there was no conflict of interest in the present study.

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Abbreviation

- *DTS*: Diametral Tensile Strength
- *EPS*: Extracellular Polymeric Substances
- *MIC*: Minimum Inhibitory Concentration
- *RCT*: Randomized Controlled Trial

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REVIEW ARTICALE

EVALUATING THE EFFICACY OF DICLOFENAC IN MANAGING POSTOPERATIVE SEQUELAE AFTER MANDIBULAR THIRD MOLAR EXTRACTION - A SYSTEMATIC REVIEW AND META-ANALYSIS

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ABSTRACT

Objectives: Postoperative pain following mandibular third molar surgery is a prevalent and often debilitating condition that necessitates targeted pain management. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed to alleviate this discomfort. This review aims to provide an updated synthesis of the current evidence on the efficacy and safety of diclofenac for managing postoperative pain after third molar surgery, by evaluating findings from relevant studies in the dental literature.

Materials and Methods: A literature search was conducted in PubMed and EBSCOhost databases until March 2025. The present systematic review assessed the effectiveness and safety of diclofenac for managing postoperative pain following third molar removal. Twenty-eight studies were included for review based on PRISMA guidelines. The review encompassed randomized controlled trials and clinical trials that examined postoperative pain management after mandibular third molar surgery.

Results: The present systematic review and meta-analysis included 24 studies. For statistical analysis, six study data for visual analogue scale (VAS) pain score were used. The pooled effect size of VAS pain scores was statistically not significant when compared to diclofenac versus other NSAIDs.

Conclusion: Diclofenac is a key analgesic for postoperative dental pain and has a favorable safety profile and efficacy. However, further research is needed to assess its long-term safety and efficacy in chronic pain management

Keywords: Diclofenac, efficacy, safety, postoperative pain, mandibular third molar

1. INTRODUCTION

From time immemorial clinical dental practice has relied heavily on the use of non-steroidal anti-inflammatory drugs (NSAIDs) for pain management. Many drugs have been employed over the years with varying degrees of success in postoperative pain management.

The postoperative sequelae of third molar surgery are associated with pain, swelling and reduced mouth opening. Most patients are prescribed NSAIDs for the management of postoperative discomfort. However, each individual responds differently to the use of NSAIDs.

Over the years, combination therapy has become prevalent due to better postoperative outcomes.[1] NSAIDs are often prescribed in combination with anti-inflammatory enzymes or steroids to improve patient comfort and recovery.[2,3]

Effective pain management is crucial for improving postoperative outcomes and patient satisfaction. Diclofenac, a widely used NSAID, has been extensively studied for its role in alleviating postoperative dental pain. Diclofenac is a phenylacetic acid derivative product of rational drug design based on phenylbutazone, mefenamic acid, and indomethacin structures.[4] Structurally, two chlorine groups are present in the ortho position of the phenyl ring, locking the ring in maximal torsion that appears to be related to increased potency.

Diclofenac is known to exert its antinociceptive effect by inhibition of cyclooxygenase - 1 and - 2 enzymes responsible for producing prostaglandins.[5] Most of the adverse drug events associated with diclofenac are attributed to its mechanism of action. Clinicians are always wary of the serious gastrointestinal, cardiovascular and renal side effects that impact the outcome of therapy. Advances in pharmacotherapeutics have led to the alteration of the pharmacokinetic properties of oral diclofenac drug products that have several desirable characteristics, like convenient dosing, improved absorption, and rapid onset of analgesia.

With the advent of novel technology, drug particle size has been reduced for the development of low-dose oral diclofenac with good analgesic efficacy and considerably reduced systemic exposure.[6]

Properties of topical as well as injectable diclofenac have also been improved for a wider therapeutic range.[7]

1.1 Rationale

This review adopts an innovative narrative approach by synthesizing evidence from recent studies to provide a comprehensive assessment of the efficacy and safety profiles of various diclofenac formulations. It systematically evaluates how different dosing regimens and routes of administration influence clinical outcomes. Additionally, the review contextualizes diclofenac's performance in comparison to other non-steroidal anti-inflammatory drugs (NSAIDs), highlighting both its advantages and limitations.

This analysis aims to identify existing knowledge gaps, particularly regarding the long-term effects of diclofenac in diverse patient populations and the impact of novel formulations on therapeutic outcomes. By addressing these gaps, the review seeks to inform dental practitioners and guide future research directions in the field of dental pain management and associated postoperative inflammatory conditions.

1.2 Objectives

The objective of this systematic review was to systematically map and evaluate the available evidence on the safety and efficacy of diclofenac in managing postoperative pain following third molar surgery. Specifically, this review aims to:

1. Assess the effectiveness of diclofenac in reducing pain intensity and improving recovery outcomes after third molar removal.
2. Examine the safety profile of diclofenac, focusing on the incidence and severity of adverse effects.
3. Compare the efficacy and safety of diclofenac with other commonly used analgesics in the postoperative management of third molar surgery.
4. Identify knowledge gaps and suggest areas for future research regarding the use of diclofenac in this clinical context.

1.3 Research question

The research question was formulated whether the diclofenac shows higher efficacy and safety profile over the other NSAIDs in the management of postoperative pain after third molar surgery. The PICO structure of the research question was as follows:

Participants – Patients with symptomatic third molars

Intervention – Surgical extraction of third molars under local anesthesia

Comparison – Comparison with other NSAIDs and/or placebo

Outcomes – Efficacy of diclofenac over other NSAIDs

2. MATERIAL AND METHODS

2.1 Protocol and Registrations

The protocol of the present systematic review and meta-analysis was registered on PROSPERO international prospective of systematic reviews under registration number CRD420250651242 on 24th February 2025.

2.2 Eligibility criteria

The present systematic review followed the PRISMA guidelines for the assessment of the efficacy of diclofenac in managing postoperative sequelae after mandibular third molar extraction. The articles searched on the databases were sorted based on the following exclusion and inclusion criteria:

2.2.1 Inclusion Criteria:

1. Study Type: Randomized controlled trials / Clinical trials
2. Language: Articles published in English.
3. Population: Human studies on the adult population (age more than 18 years) undergoing third molar surgery.
4. Intervention: Studies in which diclofenac was administered orally or parenterally as the primary pharmacological intervention for postoperative pain management.

5. Outcome Measures: Efficacy (e.g., pain relief, anti-inflammatory effects) and safety (e.g., gastrointestinal, renal, and cardiovascular adverse effects), comparisons of diclofenac with other NSAIDs and placebo, and variable dosing forms of diclofenac.

2.2.2 Exclusion Criteria

1. Study design: Studies that used split-mouth design for comparison of various drugs.
2. Intervention: Studies where diclofenac was used in combination therapies without clear delineation of its specific effects.

2.3 Information sources

We conducted a deep search of PubMed/MEDLINE and EBSCOhost databases till 3rd March 2025. The periodic search was continued during the preparation of the manuscript so as to find any new publications across the databases.

2.4 Search strategy

The search terms "Diclofenac", "postoperative dental pain", and "third molar extraction or surgery" were used for article data retrieval under the search schema of "All fields" in the query box of the advanced search engine on PubMed/Medline and EBSCOhost. The Boolean operator provided for the search exhibited as ((diclofenac) AND (postoperative dental pain)) AND (third molar extraction or surgery)). Clinical trials were only included after using advanced filter. Articles published in English language only were screened for review.

2.5 Selection process

A total of 115 studies were retrieved after the deep search of the PubMed/Medline database and 152 studies were retrieved from EBSCOhost. Studies swotted by the authors for the content, search keywords and relevance for the present review. Initial scrutiny yielded 57 clinical trials / randomized controlled trials. Thus, 57 articles were analysed for content and relevant data to study the efficacy of diclofenac compared to other NSAIDs. Further, 33 articles were eliminated based on exclusion criteria. Finally, 24 research articles were presented for scoring and systematic review based on PRISMA guidelines (Figure 1).

2.6 Data collection process

The authors thoroughly revised the finalized 24 articles using the Scottish Intercollegiate Guidelines Network (SIGN) guidelines for article selection that follow the protocol for case-control studies.[8] All these articles fulfilled the SIGN criteria and were tabulated for analysis, data screening and inferences. These potentially relevant studies were assessed individually by three authors for eligibility, and any disagreements were sorted by re-reading and resolving the conflicts.

2.7 Data items

The primary outcome data was retrieved for assessment of pain using visual analogue scale (VAS) at least 24 hours after the surgical removal of mandibular third molar. Other parameters like postoperative swelling, limitation in mouth opening and use of rescue analgesics were reviewed, however, the assimilated data was incoherent for the purpose of statistical analysis.

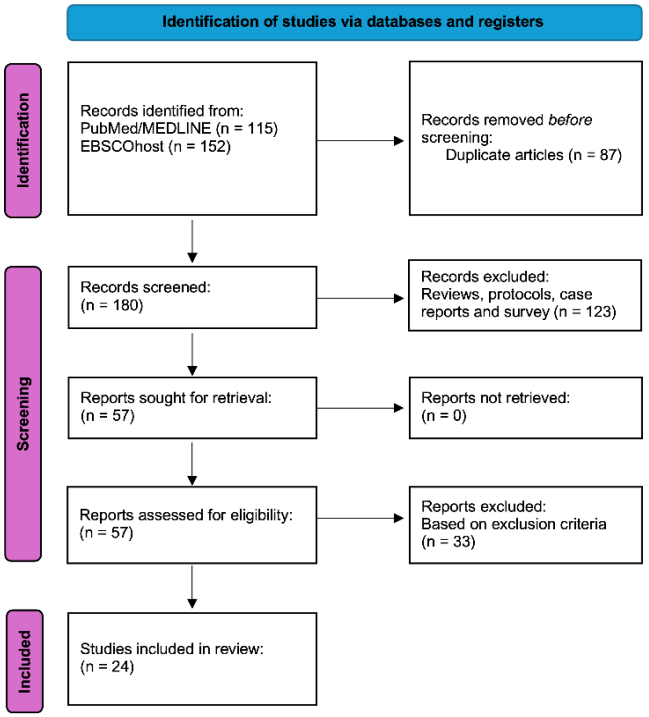


Figure 1. PRISMA Flow Diagram

2.8 Risk of biases assessment

The included articles for the review have Diclofenac as common variable while the comparison variables are different NSAIDs and placebo. Moreover, the observable biases included variations in visual analog scale and verbal rating scale scores. While few studies focused on total pain relief scores, the studies did not utilize the same standardized facial measurements for analysis of postoperative swelling. The presented data in the included studies was continuous and hence could not be analyzed by the COCHRANE Risk of Bias assessment tool.

2.9 Synthesis measures

Review manager computer program (RevMan) version 5.4 was used for data entry and calculation of effect size for VAS from six studies.[9]

The forest plot was derived by entry of VAS scores recorded for diclofenac against other NSAIDs used for comparison during third molar surgery. The qualitative syntheses for diclofenac were focused on efficacy, safety profile, effect on inflammatory mediators and other relevant findings.

3. RESULTS

3.1 Study selection

This systematic review synthesizes findings from 24 studies on the efficacy and safety profile of diclofenac in managing postoperative pain following third molar surgery. The included studies evaluated various formulations of diclofenac, including oral, injectable, transdermal, and submucosal forms, and compared them to other NSAIDs or placebo. Studies that were presented through the search engine but did not qualify in the systematic review were excluded based on the defined inclusion and exclusion criteria.

3.2 Study characteristics

The articles collected were randomized clinical/control trials, case-control, and comparative studies that fulfilled the inclusion criteria had relevance in synthesizing evidence were summarised for primary objectives and outcomes.

3.3 Results of individual studies

Efficacy of Diclofenac

Several studies have confirmed the effectiveness of diclofenac as a standalone analgesic for postoperative pain. Zuniga et al. found that liquid-filled diclofenac capsules significantly improved pain outcomes with a rapid onset of relief, observed within 30 minutes in their later study.[10,11] Similarly, Gorecki et al. reported that submucosal diclofenac effectively reduced pain following third molar surgery and required fewer rescue analgesics compared to placebo.[12]

Diclofenac was consistently found to be comparable to other analgesics in controlling postoperative pain. Orozco-Solís et al. compared 100 mg diclofenac to 15 mg meloxicam following third molar extraction and found no statistically significant differences in pain control, facial swelling, or trismus, indicating similar efficacy between the two drugs.[13] Similarly, Ahlstrom et al. found that diclofenac provided similar pain relief compared to ibuprofen (400 mg), with a comparable safety profile.[14] Shah et al. also highlighted diclofenac's superiority to placebo, showing a significant reduction in postoperative swelling and tenderness, further supporting its role in both analgesia and inflammation control.[15] Diclofenac was similarly effective across different formulations. Seymour et al. found that intravenous diclofenac in doses of 25, 50, and 75 mg provided comparable pain relief, indicating that the dose did not significantly affect the drug's efficacy.[16]

Hofele et al. found that both tablet and sachet forms of diclofenac provided effective pain management, though the sachet form offered incremental benefits in terms of analgesia.[17] Samal et al. compared the transdermal patch to oral and intramuscular diclofenac and concluded that transdermal and oral forms had similar analgesic efficacy however, the injectable form provided better pain control.[18] Diclofenac had similar efficacy to piroxicam but had a shorter duration of action and slightly lower incidence of nausea and vomiting as suggested by Wakeling et al when used as a suppository.[19] However, a study has shown a better analgesic effect, longer duration of action and fewer adverse events associated with the use of other NSAIDs tenoxicam.[20]

Safety Profile of Diclofenac

The safety profile of diclofenac has been generally favorable, with most studies reporting a tolerable adverse effect profile compared to other NSAIDs.

Common side effects of diclofenac included gastrointestinal discomfort, nausea, headaches, and dizziness, but these were generally mild and comparable to other NSAIDs. Joshi et al. found that diclofenac was associated with side effects such as nausea and gastrointestinal discomfort, but these were not significantly different from those observed with ibuprofen and paracetamol with codeine.[21] Manvelian et al. found that diclofenac (in both 18 mg and 35 mg doses) had high overall efficacy and tolerance, with treatment-related adverse effects being comparable to celecoxib.[22]

In comparative studies, diclofenac was generally found to have a safety profile similar to other NSAIDs, such as ketoprofen and ibuprofen. Tai et al. showed that diclofenac provided better pain relief than controlled-release ketoprofen, with similar adverse effects, further supporting diclofenac's favorable safety profile.[23] Gaetano et al. compared diclofenac with etoricoxib and found both drugs to be effective, though etoricoxib resulted in a statistically significant reduction in pain severity.[24] Mohammad et al. compared sublingual piroxicam with oral diclofenac (150 mg) and found that piroxicam demonstrated better tolerability and efficacy.[25] Shukla et al. also reported that piroxicam provided better analgesic properties and required fewer rescue analgesics compared to diclofenac, although other analgesics like tramadol and paracetamol were less effective.[26] MV et al. conducted a study comparing the intramuscular administration of ketoprofen and diclofenac for postoperative pain management. The results suggested that both drugs exhibited comparable efficacy in alleviating postoperative pain, leading the authors to propose that ketoprofen and diclofenac could be used interchangeably for this indication.[27]

Effect of Diclofenac on Inflammatory Mediators

Diclofenac's role in modulating inflammatory mediators, particularly its effects on C-reactive protein (CRP) levels and swelling, was investigated in several studies. Singh et al. compared diclofenac to mefenamic acid and found that both drugs were equally effective in pain relief, though mefenamic acid significantly reduced serum CRP levels compared to diclofenac.[28]

Salgia et al. also measured CRP levels and found that diclofenac caused the lowest increase in CRP among the NSAIDs tested, further suggesting that diclofenac may have a milder impact on systemic inflammation than some other NSAIDs like mefenamic acid.[29]

Effect of Diclofenac on other postoperative sequalae

Diclofenac's anti-inflammatory effects were also evident in reducing postoperative swelling and tenderness. Shah et al. found that diclofenac significantly reduced postoperative swelling and tenderness compared to placebo, indicating its efficacy in managing local inflammation.[15] Similarly, Orozco-Solís et al. found that diclofenac provided comparable effects to meloxicam in controlling facial swelling and trismus after third molar extraction, highlighting its role in managing both pain and inflammation.[13]

Kaplan et al. compared diclofenac to tenoxicam and flurbiprofen, noting that while tenoxicam was superior in terms of pain control, diclofenac was still effective and its anti-inflammatory properties were evident.[30] Additionally, Mony et al. assessed the preemptive analgesic effects of diclofenac and ketorolac, concluding that both medications significantly provided postoperative pain-free periods, suggesting that diclofenac's anti-inflammatory properties may contribute to its analgesic effects.[31]

Furthermore, a study highlighted the role of diclofenac in reducing inflammation, suggesting that only 30% of patients receiving diclofenac required rescue analgesics, suggesting good control over both pain and inflammation.[32]

Other relevant findings

Hersh et al. studied the dose-ranging of Prosorb diclofenac potassium (25, 50, and 100 mg) and found that all doses were more effective than placebo, with rapid onset and good tolerability, further supporting the efficacy of diclofenac in managing postoperative pain and inflammation.[33]

Results of syntheses

Six studies provided the relevant data for sample size and mean pain scores with standard deviations (SD).[25 – 27, 29, 31, 32]

The forest plot analysis was performed for these studies using RevMan for academics.[9]

The synthesis calculated the pooled average effect size, SD, at a 95% confidence interval (CI). The analysis revealed a nonsignificant comparison between pooled pain scores of diclofenac versus other NSAIDs (p-value = 0.82, effect size =0.03, and 95% CI range = -0.23 to 0.29). Forest Plot of pooled effect size, estimates and 95% confidence interval representing differences in VAS scores for the six studies (Figure 2).

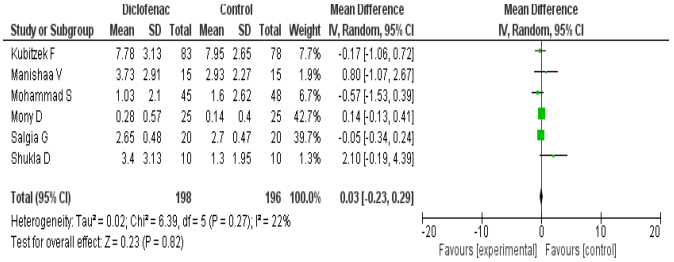


Figure 2. Forest Plot of pooled effect size, estimates and 95% confidence interval representing differences in VAS scores

3. DISCUSSION

Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) belonging to the carboxylic acid class and is a phenylacetic acid derivative. It exerts its analgesic effects by inhibiting cyclooxygenase (COX) enzymes, key players in the arachidonic acid pathway responsible for prostaglandin synthesis. By blocking COX, diclofenac reduces the inflammation and pain that arise post-surgery, making it particularly effective in managing postoperative dental pain after oral procedures such as third molar extractions.[16] These surgeries offer a standardised model for evaluating analgesic efficacy due to their consistency and the availability of healthy individuals as study subjects. Numerous studies have demonstrated the efficacy of NSAIDs in alleviating dental pain, and diclofenac, a widely used agent in the field, has shown significant analgesic potential. However, its recommendation has waned recently, raising questions about its current standing in pain management protocols.[34] This systematic review includes 24 studies examining diclofenac's efficacy, tolerability, and safety in managing postoperative dental pain. The evidence consistently supports diclofenac as a highly effective analgesic for postoperative pain, particularly in dental and third molar surgeries. Its efficacy stems from inhibiting COX enzymes, reducing prostaglandin production, which mediates pain and inflammation. The findings reinforce diclofenac's continued relevance in pain management, with studies demonstrating its comparable or superior efficacy to other common analgesics, such as meloxicam, tramadol, ibuprofen, and paracetamol. In submucosal formulations, diclofenac demonstrated a faster onset and reduced need for rescue analgesics, improving patient comfort post-surgery.[12]

Additionally, when compared to other NSAIDs such as ibuprofen, ketoprofen, and piroxicam, diclofenac is shown to be at least comparable, if not superior, in terms of pain relief and rescue analgesic consumption. [26]

However, when compared to etoricoxib, a selective COX-2 inhibitor, diclofenac performed less favorably in certain cases. Etoricoxib may offer superior pain relief with potentially fewer gastrointestinal side effects due to its selective inhibition of COX-2, rather than the

non-selective COX inhibition seen with diclofenac. This suggests that newer COX-2 inhibitors may have a role in pain management with fewer adverse effects, particularly in patients with a higher risk of gastrointestinal irritation. This highlights the importance of personalized treatment based on patient characteristics and the clinical scenario.[24] Further studies comparing various delivery routes (oral, intravenous, submucosal) also indicate consistent efficacy across formulations. Another study found that intravenous diclofenac, regardless of the dose (25 mg, 50 mg, or 75 mg), provided comparable pain control.[16] Oral tablets and soft gel capsules also showed similar efficacy, with liquid-filled capsules offering a faster onset, beneficial for acute pain management.[10, 11]

Diclofenac also demonstrates its dual role in pain relief and inflammation modulation. Studies show that it reduces inflammatory markers like CRP more effectively than other NSAIDs, such as mefenamic acid, which is especially significant in postoperative care, where inflammation is a key factor in pain perception and healing.[28, 29] This may provide added advantages over other NSAIDs by reducing both pain and inflammatory sequelae more effectively.

4.1 Limitations and Future Directions

Despite diclofenac's effectiveness, several limitations must be considered. Many studies in this review had small sample sizes and primarily assessed short-term outcomes (e.g., pain within the first few days after surgery). These limitations hinder the ability to conclude the long-term safety and efficacy of diclofenac. Future studies should include larger sample sizes and evaluate long-term outcomes, particularly concerning gastrointestinal, renal, and cardiovascular effects—common concerns with prolonged NSAID use. Most studies in this review have not specified the type of trial in methodology.

Thus, also leading to issues with justification for selection of comparing drug.

Another limitation is that most studies did not assess inflammatory markers or other biomarkers of healing, even though inflammation plays a critical role in postoperative recovery. Future studies could benefit from including serum CRP levels or other inflammatory biomarkers to better understand diclofenac's impact on inflammation.

Moreover, the findings of this review primarily focus on third molar extractions, a standardized surgical procedure, which may not be fully applicable to other surgical types or more diverse patient populations.

Additionally, while diclofenac is widely used in clinical practice, the absence of head-to-head comparisons with newer COX-2 inhibitors or other non-opioid analgesics leaves its relative position in pain management protocols unclear. Future research could directly compare diclofenac with emerging agents to help establish clearer guidelines for postoperative pain management across a variety of surgical settings.

3. Summary and conclusion

This systematic review underscores the pivotal role of diclofenac as an effective NSAID in managing postoperative dental pain, particularly following third molar extractions. The synthesis of evidence from the selected studies reveals that diclofenac has comparable analgesic efficacy and demonstrates a favorable safety profile to other NSAIDs.

However, the review also highlights significant gaps in the current literature, particularly concerning the long-term effects of diclofenac in diverse patient populations and the need for standardized assessment criteria that incorporate inflammatory markers. While the majority of studies focus on short-term outcomes, the implications for chronic pain management remain underexplored. Furthermore, the findings are primarily relevant to adult patients undergoing third molar surgery, potentially limiting their applicability to broader surgical contexts or pediatric populations.

In conclusion, while diclofenac remains a cornerstone in the arsenal of postoperative pain management, continued research is essential to address the identified gaps and optimize its use across various clinical scenarios. The need of the hour is to develop standardized guidelines and protocol for selection of analgesics for specific outcome and the procedures and not merely relying on clinicians' experiences.

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Ethical Approval

Not applicable

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Not Applicable

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REVIEW ARTICLE

RANK LIGAND-OSTEOPROTEGRIN SYSTEM AS A DIAGNOSTIC TOOL IN OSTEOPOROSIS

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ABSTRACT

Background: Osteoporosis is a systemic disease of the skeletal system, characterized by low mineral density and the deterioration of the micro-architecture of the bone tissue, with a consequent increase in bone fragility which has a significance for dentists and TMJ specialists as well.

Aim: The purpose of this study was to analyze the importance of the Receptor Activator of Nuclear (RANK) ligand-osteoprotegerin system in bone metabolism and evaluate what may be predicting factors of early diagnosis while taking into consideration the impact of gender.

Methods: In this study diagnostic strategies are proposed in situations predisposing to osteoporosis. The RANK ligand-osteoprotegerin system could be an excellent tool for prevention.

Conclusion: Prevention remains the best strategy in avoiding the development of this disorder which can be disabling but also it could be leading to lethal consequences if neglected. The use of specific screenings such as those using RANK-ligand osteoprotegerin can be useful to reduce the onset of the disease. Furthermore, due to its significantly different impact in males vs. females.

Key words: rank ligand system, osteoprotegerin, menopause, gender medicine, bone mineral density, periodontitis and dental movement, osteoporosis, metabolism bone

INTRODUCTION

Osteoporosis is one of the main metabolic pathologies of the bone characterized by bone loss and de-mineralization and although it is mistakenly considered a pathology of the elderly, it can also be found in young subjects as well[1]. Estrogen deficiency leads to a reduction in bone mineral density in women, during both fertile years (hypoestrogenic amenorrhea) and during menopause, while androgen deficiency induces an increase in RANKL for men also, with consequent loss of bone mass [2].

The predisposing factors are many: family history of osteoporosis, early menopause, low testosterone levels, amenorrhea, anorexia or bulimia, thyroid disease, rheumatoid arthritis, diseases involving blocked intestinal absorption of calcium, use of corticosteroid drugs, use of anticonvulsant drugs, low-calcium diet, lack of exercise, smoking, consumption of alcohol, unhealthy eating habits [3].

Osteoporosis is a disease that cannot always be diagnosed in time, it is often diagnosed after a fracture (the most frequent hip, vertebra, wrist fractures or a condyle fracture) and for this reason prevention screening must not be neglected. It is estimated that 40% of elderly women and 15% of elderly men are prone to skeletal fractures [4-6].

According to data from the World Health Organization (WHO), osteoporosis affects more than 75 million people in the United States, Europe and

Japan. In the United States and the European Union, about 30% of postmenopausal women have osteoporosis and it is estimated that more than 40% will have a fracture sometime after menopause. It is estimated that about 4 million women in Italy have osteoporosis [8,9]. For a man, the risk of suffering a fracture over the course of his life can be up to 27% more in those over 50s, which is higher than the risk of developing prostate cancer (11.3%), but few people are aware of it. As for women, the mortality rate after a hip fracture increases with age, and it is higher in the 12 months after the event. However, in the first 6 months. In general, although fragility fractures are more common among women, mortality rates following a fracture are higher in men and they are more likely to suffer serious consequences after the event. [11,12].Men, therefore, should be aware of their risk factors and try to prevent the disease, by reducing modifiable risk factors (such as proper nutrition) and manage those that cannot be modified[13].The risk factors for osteoporosis in men are largely comparable to those in women: testosterone deficiency (primary or secondary hypogonadism) and the possible use of androgen deprivation therapy (ADT), a treatment used in some cases to fight prostate cancer. In the presence of these risk factors, a man between 50 and 69 years old should undergo a DEXA test to assess whether he has osteoporosis. The examination to evaluate bone density should be performed in any case once age 70 is reached and in particular if the man has already suffered a fracture following a fall after age 50, if he is being treated with steroids or ADT, and if he has low testosterone levels.

Diagnosis is currently made with laboratory and diagnostic tests, such as the computerized bone mineral density (BMD) test. There are many examination instruments for the study of bone computed tomography (CT), scintigraphy and positron emission tomography/computed tomography (PET/CT)[7]. Several studies support the application of ^{18}F-sodium fluoride (NaF) PET-CT to evaluate benign bone diseases, although its clinical use is currently limited to the detection of skeletal metastates. In particular bone turnover can be measured through NaF uptake to diagnose osteoporosis and evaluate the efficacy of therapeutic treatment with bisphosphonates 5 .But despite being a disabling disease and with serious consequences, usually it is diagnosed late: during menopause in women and often only after the fracture in men. Estrogen deficiency negatively affects other structures as well, purview of the dental profession, such as the alveolar bone, oral mucosa, and salivary glands [13].

This review aims to describes the RANK-ligand (RANKL) osteoprotegerin (OPG) as a screening test for the evaluation of bone metabolism, to prevent conditions such as osteoporosis.

The limitation of this review is the scarce number of studies in the literature on this topic.

Nevertheless, we aim to draft the guidelines to carry out the analysis of bone metabolism to prevent a late diagnosis.

Factors affecting bone metabolism

Gender studies

The gender studies in relation to bone metabolism are particularly interesting. It is known that men have a greater bone mass than women, making osteoporosis less frequent in them, with a ratio of 1:4 for women and 1:10 for men. This data has concentrated the focus mostly on female osteoporosis both in terms of diagnosis and in terms of prevention. Many more women take calcium and vitamin D supplements in menopause and there are many more therapeutic strategies for women, starting with the correction of estrogen deficiency in menopause with hormone replacement therapy. Unfortunately, the so-called "gender blindness" has impacted males and so inevitably there are a greater number of men with undiagnosed advanced osteoporosis. About 20% of all hip fractures occur in males and the incidence of vertebral fractures is about half that of females. However, mortality and morbidity from vertebral and femoral fractures are higher in males than in females. Therefore, it is necessary for both women and men to find a way to diagnose osteoporosis as early as possible [18, 19].

Besides laboratory tests [calcium (Ca), phosphorus (P), calcitonin (CT), parathyroid hormone (PTH), 25OH Vitamin D, osteocalcinin (OC), pyridinoline or telopeptide (CTX)] and radiological tests (DEXA), a specific system to evaluate the metabolism of the bone is proposed. The dentist can represent the sentinel doctor because he often has to request orthopanoramic radiological examinations where the quantity of bone can be highlighted [15, 17].

Estrogens have a protective effect on bone, and there are both in vitro and in vivo studies that show that these effects are mediated by the RANKL/OPG system. In particular, estrogens increase the production of OPG in osteoblastic cells in vitro [16], and in young women who use oral estrogen-progestins the serum levels of OPG are significantly increased compared to a control group consisting of young women who did not use it [16]. This increased free OPG/RANKL ratio seems to justify the positive effect of estrogen-progestogens on the skeleton [20, 21].

Drugs that interfere with bone metabolism

There are numerous drugs that interact with bone metabolism through three main mechanisms

1. increased osteoclast activation and induction of high bone turnover

2. direct suppression of osteoblastic new bone formation
3. inhibition of normal osteoid mineralization

Drug-induced bone loss can occur with varying frequency but it can be prevented or treated if the potential osteopenic effects that these drugs exert are known, which are altering the homeostasis of calcium, vitamin D and PTH, local factors and endocrine and paracrine hormones.

The use of these drugs, by reducing bone mass, would cause fractures, therefore it is important to recommend prudent use of the following drugs, using the minimum effective doses and/or limiting their administration to the strictly necessary time frame[17=19].

MATERIAL AND METHODS

This study received approval from the Ethic committee of Cattolic University Rome(n.30759/19) ID : 2700

In this study we propose diagnostic strategies in situations predisposing to osteoporosis. The RANK ligand-osteoprotegerin system could be an excellent tool for prevention of osteoporosis [28-30].

Role of RANKL/OGP system

In a complex system such as that of bone metabolism in which calcium, magnesium, vitamin D and the hormones estrogens, androgens, calcitonin and parathyroid have an important role, the RANKL/OPG has been emerging as a fundamental physiological system in recent years [9]. Under physiological conditions, the balance between bone resorption and neo-apposition is regulated by the expression of two proteins: the RANKL (Receptor Activator of Nuclear NF-κB Ligand, secreted by activated T and B lymphocytes and osteoblasts) and the OPG (osteoprotegerin, secreted by fibroblasts of the periodontal ligament and osteoblasts) (Lu et al., 2006)[5]. Basically, OPG prevents RANKL from binding to its true receptor (RANK), creating a real competitive inhibition most of the secreted RANKL is saturated by OPG, which constitutes a false target for RANKL, while a small fraction of RANKL interacts with its natural receptor RANK (Receptor Activator of Nuclear Factor Kappa B) present on osteoclastic precursors, with consequent maturation of the osteoclast and bone resorption. In the presence of inflammation, the secretion of inflammatory cytokines causes activated T and B lymphocytes and osteoblasts to produce massive amounts of RANKL [10]. Thus, there is an up-regulation of RANKL associated with a down-regulation of OPG which will be produced in minimal quantities. The RANKL produced in excess, will therefore bind the RANK present on the osteoclastic precursors, resulting in substantial bone resorption. The same unbalanced mechanism towards bone resorption occurs in menopause causing osteoporosis. In fact, estrogen deficiency determines an activation of bone remodeling processes, in which osteoclastic activity leads to an increase in bone resorption processes, not balanced by an adequate increase in osteoblastic functions, with a significant reduction in typical bone mineral density [10].

In addition to the serum level, the RANKL/OPG system has also been detected in gingival tissue, crevicular fluid and in saliva in various studies published in the last 10 years. For example, an altered ratio of the RANKL/OPG system, with an increased expression of the mRNA for RANKL, has been demonstrated in the gingival tissues of patients suffering from periodontal disease associated with local osteolysis [29-32].

It is an immunoenzymatic assay for the measurement in biological fluids, such as serum and saliva, of a protein that uses a specific antibody to detect the substance of interest in the biological fluid tested and a colorimetric enzymatic reaction produced by an enzyme linked to the antibody used that can be read and therefore quantified with a spectrophotometer, the intensity of the color will be directly proportional to the total concentration in the sample of the substance of interest.

DISCUSSION

The details of bone metabolism still require a lot of in-depth studies and we consider a great limitation that often the study of bone metabolism is done only in some conditions such as menopause.

However, it would be desirable to do so too in amenorrhea or fractures. Meantime, although laboratory and other tests are available, the diagnosis of osteoporosis is often late. MOC is recommended in all subjects between 50 and 69 and currently the densitometric criteria for the diagnosis of osteoporosis in males are not based on levels of evidence equal to those for females.

The goal is to prevent osteoporosis in the future, so it is necessary that all specialists cooperate in diagnosing bone dysfunction as early as possible. Even the dentist can notice local signs and symptoms of osteoporosis, thanks to a study by Taguchi (2015) on menopausal women suggesting that the orthopanoramic is a sufficiently sensitive investigation tool to draw a diagnosis of osteoporosis, therefore allowing the dentist often to be the first to notice bone defects. Plus tooth displacements may suggest bone metabolism problems [34-36].

Proposal for future studies

If much is known about the behavior of the femoral and vertebral bones in conditions of

hypoestrogenism, on the other hand the data relating to the behavior of the bones of the oral cavity are decidedly scares. Only in recent years have specific studies been carried out to investigate the pathophysiological mechanisms that regulate the functions of the oral cavity in this phase of life. The available data indicate that sex hormones play a role in the physiology of oral mucosa, the salivary glands and the bones that support the teeth, and there is also a clear deterioration in the state of oral health in menopause which has a significant impact on the quality of life of the woman [20-23].

Several markers of bone remodeling have been used so far to study bone metabolism in menopause, but in recent years the RANK/OPG system is assuming an increasingly important role in this area 21-24.

This system regulates the balance between bone resorption and neo-positioning. Most of the secreted RANKLof bone remodeling have been used so far to study bone metabolism in menopause, but in recent years the RANKL-OPG system is assuming an increasingly important role in this area 21-28). This system regulates the balance between bone resorption and neo-positioning. Most of the secreted RANKL (NF-kB Receptor Activating Ligand, secreted by activated T and B lymphocytes and osteoblasts) is saturated by OPG (Osteoprotegerin secreted by fibroblasts, also of the periodontal ligament, and osteoblasts) which is a false target of RANKL, the unbound portion interacts with its natural receptor RANK, present on osteoclast receptors, resulting in osteoclast maturation and bone resorption. Moreover, this system appears to be modulated by estrogens. Several studies have highlighted the presence of this system in different tissues of the oral cavity, trying to define its role both in physiological and pathological conditions or during tooth movement induced with orthodontic treatments(38,39,40) The role of sex hormones in influencing the health of all oral tissues and the positive role of HRT in the oral area in menopause, as well as the pathophysiological mechanisms that determine these conditions are not yet well defined [33,34]. Therefore, we need to acquire new data in order to be able to formulate recommendations regarding the promotion of oral health in menopausal women [25- 32].

Furthermore, for males it is necessary to make an early diagnosis for example by screening for osteoporosis all the patients who have used corticosteroids for extended periods of time to control asthma or other disorders [35-37].

The role of the dentist is very important to evaluate bone metabolism and can be a reference figure as a sentinel doctor for the early recognition of these problems: the oral cavity is affected by the hormonal impact in fact the estrogen deficiency negatively affects the alveolar bone, the oral mucosa the salivary glands and with menopause it is possible to find an alteration of periodontal health with xerostomia, increased incidence of caries, atrophic gingivitis, periodontitis, resorption of alveolar bone, crowding or opening of spaces (diastemas) and dental movement during orthodontic treatment requires active bone remodeling, and that the speed of movement is inversely proportional to bone density, estrogen decreases the rate of tooth movement during orthodontic treatment, in fact young girls under PE show a reduction in orthodontic movement [24]. In the future, it is desirable that the dentist who normally sees patients for periodic checks in the event of alterations in the oral cavity advises the patient for an in-depth diagnostic study [41].

CONCLUSION

Although the medical literature is still very lacking on this subject, from the results of the current investigation, future studies will give the possibility of new early diagnosis and treatment of osteoporosis. Another important aspect is that in the field of gender medicine, males are less favored in the diagnosis of osteoporosis, therefore it is necessary to sensitize patients regarding the need to carry out diagnostic screening, while research on the impact of osteoporosis on non-binary gender is currently absent. Rank-ligand studies would open new frontiers on the study of bone metabolism and in the prevention of osteoporosis. Prevention remains the best weapon in avoiding the development of this disorder which can be disabling but also it could be leading to lethal consequences if neglected. The use of specific screenings such as those using RANK-ligand osteoprotegerin can be useful to reduce the onset of the disease.

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Conflict of interest
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CASE REPORT

FROM FRACTURE TO AIRWAY THREAT: A CASE OF LUDWIG'S ANGINA FOLLOWING MANDIBULAR FRACTURE

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ABSTRACT

Background: Ludwig's angina is a rapidly progressive, potentially fatal cellulitis of the floor of the mouth and neck, often arising from odontogenic or mandibular infections. Prompt surgical intervention and appropriate antimicrobial therapy are critical for successful outcomes.

Case Presentation: A 53-year-old male presented to the emergency department with severe facial swelling, trismus, dysphagia, and signs of airway compromise. Clinical and radiographic evaluation revealed an untreated, intraorally open mandibular fracture complicated with Ludwig's angina, necessitating urgent surgical intervention.

Management and Outcome: Under general anesthesia, bilateral submandibular stab incisions were made for Surgical Decompression, debridement, and irrigation with gentamicin and metronidazole, followed by placement of corrugated drains, and then ORIF of mandibular and maxillary fractures was performed. Postoperatively, the patient received intravenous antibiotics, corticosteroids, NSAIDs, and fluid support. Microbiology report revealed diphtheroids on POD-4, and Gentamicin was added to drug regimen. Drains were removed on POD-5, surgical closure of the stab incisions was done on POD-12 following the resolution of infection, and the patient was discharged.

Conclusion: This case underscores the importance of early surgical intervention, multidisciplinary care, and adaptive antimicrobial strategies for managing complex facial trauma complicated by deep space infections, such as Ludwig's angina.

Keywords: Ododntogenic infections, Ludwig's angina, Mandibular fracture, Surgical intervention

1.INTRODUCTION

Maxillofacial trauma represents a significant portion of emergency presentations globally, with mandibular fractures being among the most commonly encountered injuries. These fractures often result from road traffic accidents, assaults, or falls and may be associated with open wounds that create a conduit for microbial invasion[1]. If left untreated, open mandibular fractures can lead to serious infections, including deep neck space

infections, such as Ludwig's angina.[2]

Ludwig's angina is a rapidly progressing, bilateral cellulitis involving the submandibular, sublingual, and submental spaces. It can result in airway obstruction, sepsis, and death if not promptly addressed. This condition is frequently odontogenic in origin but may also arise secondary to facial trauma, particularly when compounded by delayed or inadequate medical intervention.[3,4]

This case report describes a rare presentation of Ludwig's angina resulting from an intraorally open mandibular fracture that remained untreated for several days. It highlights the importance of early diagnosis, aggressive surgical management, and culture-directed antibiotic therapy to prevent life-threatening complications. The surgical and medical strategies adopted in this case provide valuable insights into managing similar complex presentations in clinical practice.

Case Presentation

Chief Complaints: A 53-year-old patient was reported to the Emergency Department, Yenepoya Medical College and Hospital, Mangalore, Karnataka, India with chief complaints of pain in the left upper and lower jaws following a road traffic accident on 23/03/2025. Patient reported a history of 2 episodes of Vomiting and Nasal bleeding. No history of LOC and seizures was noted. Suturing of the laceration over the upper and lower lip was done. MDCT scan was done.

Examination: Upon presentation on 23/03/2025, the patient exhibited signs of systemic compromise, including tachycardia (heart rate: 105 beats per minute), low-grade fever (100° F), and dehydration, although no respiratory issues were noted.

A clinical examination revealed significant swelling in the neck, with bilateral involvement of the submental, sublingual, and submandibular regions. The overlying skin appears stretched, shiny, and congested, with cyanosis noted in the left submandibular and submental regions (Figure 1).

Intraoral examination revealed restricted mouth opening with an interincisal distance of 15 mm. Ecchymosis was noted over the floor of the mouth (Coleman's sign) (Figure 2). In the fracture focus, there was an intraoral open wound, whose instrumental exploration evidenced bone stumps in the fracture area. Occlusion was deranged and avulsion was noted with respect to 11,12, 21, 31, 32, 41 and 42 teeth. Sutured laceration was noted.



Figure 1. Shiny, stretched skin noted over the left submandibular and submental regions.



Figure 2. Coleman's sign

Radiographic Investigation: An MDCT was performed, which showed a displaced mandibular symphysis fracture and left maxillary buttress and sinus wall fracture. It also revealed a small collection within the floor of the mouth, and significant soft tissue swelling in the submental and submandibular region (Figure 3,4).



Figure 3. Axial CT cut showing symphysis fracture.



Figure 4. 3D CT showing symphysis fracture and left maxillary buttress and maxillary sinus fracture.

Treatment: The patient was admitted as an emergency case, and surgery was carried out under general anesthesia with nasotracheal intubation. Patient was painted and draped under all aseptic precautions. Stab incision was placed 1 cm below the lower border of the mandible on both sides in the submandibular region and a pus swab was taken and sent for culture and sensitivity. The blunt dissection of the underlying tissues was performed and the submental and sublingual regions were approached contralaterally. Secretions were collected from the septic focus and were sent for microbiological examination. After draining the pus, a wash was performed with Gentamicin and Metronidazole. Then, a corrugated rubber drain was placed and secured with suture (Figure 5). After the management of Ludwig's angina, fracture management was performed.



Figure 5. Corrugated rubber drain placed and secured with suture.

Ivy eyelets were placed. Crevicular incision was placed extending from 33 to 43. Mucoperiosteal flap was raised. Subperiosteal dissection was done and fracture segment was exposed, debridement was done. Reduction of the fracture segment was reduced, and IMF was achieved. Plating was done using miniplates fixed as per Champy's line of osteosynthesis (Figure 6).

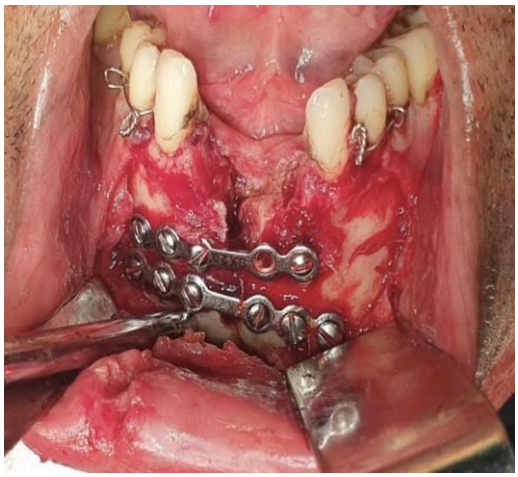


Figure 6. Reduction of mandibular fracture segment and miniplate fixation.

After plating IMF was released, and occlusion was checked.

Upper vestibular incision was placed extending from 13 to 26 region. Mucoperiosteal flap was raised. Subperiosteal dissection was done and the infraorbital and zygomatic buttress regions were exposed. Fracture fragment was reduced.2-mm 2 hole with a gap plate was fixed with 2x8 mm screws lateral to the pyriform aperture, and a 2-mm 4-hole L-shaped plate with a gap was fixed over the zygomatic buttress region with 2x8mm screws (Figure 7).

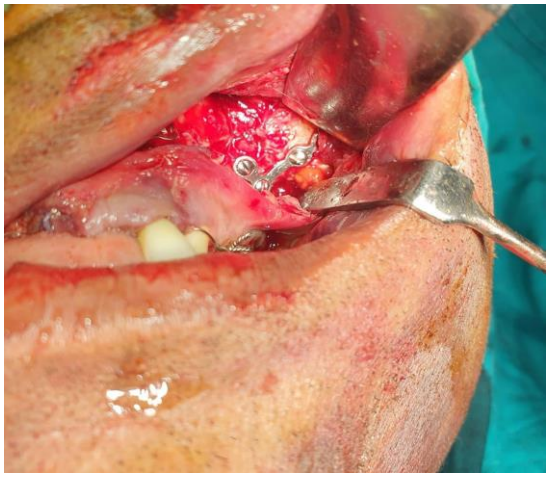


Figure 6. Reduction of maxillary fracture segment and miniplate fixation.

Hemostasis was achieved. Closure of maxillary vestibular incision and mandibular crevicular incision were closed using 3-0 vicryl. Patient extubated and shifted to the postoperative room.

Surgery was paralleled by drug treatment with Inj. Taxim 1 Gm i.v TID and Inj. METROGYL 500 Ml i.v TID. Nonsteroidal anti-inflammatory treatment was administered with Inj. DICLO 75 mg i.m twice daily, and his electrolyte balance was restored by the administration of Ringer's solution and physiological serum. Inj. Dexamethasone 8 mg i.v TID was also added, and it was tapered and stopped on POD4. Every day, Metrogyl washes were done through the drain tubes 2 times a day.

Microbiological examination demonstrated the presence of diphtheroids on POD-4, following which Inj. Gentamycin 80 mg was added to the patient's treatment regimen, and Inj. Metrogyl was stopped.

Drain was removed on POD-5, following which regular dressing was done with betadine and ribbon gauze until the pus discharge subsided. On POD-12, with the diminution of fetid secretions in the septic focus, closure of the stab incisions was done with 3-0 silk suture. Following this patient was discharged with a favourable recovery, with a normal appearance of the postoperative wound and a general good state.

DISCUSSION

Ludwig's angina was first described by the German surgeon Wilhelm Friedrich von Ludwig in 1836 as a severe diffuse cellulitis that presents an acute onset and spreads rapidly, affecting the submandibular, sublingual, and submental spaces bilaterally resulting in a state of emergency because of impending airway obstruction.[5,6]

The most common cause of Ludwig's angina is an odontogenic infection, although can also be due to penetrating injury of the floor of the mouth, osteomyelitis or fracture of the jaw, tongue piercing, sialadenitis or sialolithiasis of the submandibular glands [7]. Infection can rapidly progress and can cause a number of potentially life-threatening complications, including airway obstruction, carotid arterial rupture, carotid sheath abscess or aspiration pneumonia.[8]

The presence of immunosuppressive co-morbidities, most commonly DM, hepatic or hematological disorders have been found to be implicated in the development of severe space infections[8], and these must therefore be taken into consideration. In particular, DM has been implicated in poor wound healing and prolonged length of inpatient stay due to increased severity of infection, which is in accordance with a study by Miller EJ and Dodson TB [9]. Lack of acknowledgement around these issues can cause pitfalls in patient care, and emphasis must be placed on close monitoring, tight glycaemic control, and aggressive management of infection.

In Ludwig's angina, patients demonstrate swelling in the floor of the mouth and neck, pain, malaise, fever, and dysphagia. In these patients, inability to swallow saliva and stridor indicate imminent airway compromise. The most feared complication is airway obstruction due to elevation and posterior displacement of the tongue and edema of the glottis. Management of Ludwig's angina in this context necessitates a multidisciplinary approach.

Airway security remains a priority, and it often requires awake fiberoptic intubation or tracheostomy. Empirical broad-spectrum antibiotic therapy should be initiated early, targeting anaerobic and aerobic flora, and adjusted based on culture results. Surgical drainage, when indicated, must be performed promptly, and any focus of ongoing infection such as infected hematoma or necrotic bone must be addressed. The role of early fixation or debridement of the fracture in such cases remains a subject of debate, with some advocating for stabilization only after the resolution of infection, while others suggest early intervention to remove the nidus.[10]

In the Case reported by Mihai Juncar et al, he did extensive drainage of the affected tissues with the complete removal of necrotic areas, and fracture fixation was performed once the infection resolved after a week.[7]

In the present case, surgery proved to be a determining factor in stopping the septic process and obtaining a favourable outcome for the patient. Thus, surgical decompression and the fixation of the fracture was done at the same time. No complications were noticed in the follow-up period. Regular antibiotic washes were given twice a day. Healing of the fracture was not compromised, and two separate GA procedures were also avoided by simultaneous decompression and fracture fixation at the same time.

Our findings underscore the importance of high clinical suspicion in cases of mandibular fracture, particularly those with delayed presentation, poor dental status, or signs of systemic infection. Routine screening for signs

of fascial space involvement in such patients can facilitate early diagnosis and timely intervention. Moreover, prophylactic antibiotic coverage and meticulous oral hygiene in fracture cases, especially when surgery is delayed, may reduce the risk of progression to deep neck infections like Ludwig's angina.

CONCLUSION

Timely identification of mandibular fractures, vigilant clinical observation, proactive management of comorbidities, and appropriate antimicrobial intervention are critical for preventing severe complications such as Ludwig's angina. Delayed presentation, as observed in this patient, compounded by a new diagnosis of diabetes mellitus and resulting immunosuppression, can significantly prolong recovery and complicate treatment. We recommend early fracture recognition, judicious use of antibiotic therapy, and a high index of suspicion in patients with systemic conditions to mitigate adverse clinical outcomes and support optimal recovery.

DECLARATIONS

Ethical approval and consent to participate

Not Applicable

Competing interest

The authors declare that there are no competing interests.

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CASE REPORT

METASTATIC PAPILLARY THYROID CARCINOMA IN A BRANCHIAL CYST – CASE REPORT

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ABSTRACT

A rare case of metastatic papillary thyroid carcinoma, incidentally, found within a branchial cyst was reported in a teaching hospital, Kalutara, Sri-Lanka. A 37-year-old female presented with left side lateral neck lump of 4 years duration. Physical examination revealed a solitary, palpable, painless movable neck lump. Both ultrasound scan and fine needle aspiration cytology were suggestive of a branchial cyst. Excisional biopsy revealed that it was a papillary thyroid carcinoma metastatic to/ arising from a branchial cyst. Thyroid Fine needle aspiration biopsy confirmed a left thyroid nodule with suspicious smears (Thy 4/Bethesda). Patient underwent total thyroidectomy histopathology confirmed presence of Papillary Thyroid carcinoma.

Branchial cysts are the most common lateral neck masses. Although it is a benign lesion, rarely, it is possible to find unexpected malignancy within it. During the diagnostic phase, it should be kept in mind that this could be a metastatic lesion from a distant primary or an occult thyroid primary cancer within the branchial cyst. A thorough investigation would help identification of such malignancy and early intervention gives less trouble to both patient and clinician.

Keywords: Papillary; Thyroid carcinoma; Branchial cyst

1.INTRODUCTION

Branchial anomalies are among the commonly encountered congenital neck masses and can present as cysts, sinuses or fistulae. They have no predilection to a gender and are usually presented in the first decade of life[1]. Branchial cysts (BC) can rarely harbor malignant tissue in it, either originating de novo from the cyst or that which metastasized from elsewhere[2]. Papillary thyroid carcinoma (PTC) is the most common epithelial malignancy of thyroid tissue and can frequently cause cystic metastasis in the neck[3]. Incidence of PTCs metastasing into BC are rare in literature[4]. Diagnosis of PTC in BC is usually incidental on post excision histology and distinguishing between metastatic PTC and PTC originating from ectopic thyroid tissue within BC can be difficult [5]. This case report presents a case of PTC metastasizing into a BC. This report also serves to highlight the dilemma in precise diagnosis associated with PTC within BC.

Case report

A 37-year-old, otherwise healthy female presented to the Oral and Maxillofacial Surgical unit, for the management of a left side lateral neck mass (Fig 1).



Figure 1. Pre-operative radiograph of lateral neck mass

It initially appeared 4 years back and has increased in size gradually. She did not report any episodes of pain or dysphagia or constitutional symptoms such as fever, anorexia and weight loss. On examination she was generally fit and well. There was a smooth, circumscribed, non-tender fluctuant cyst on the anterior border of left sterno-cleido mastoid muscle measuring 3 x 3 cm. No palpable lymph nodes were noted. The examination of all other systems were unremarkable.

Assessment of the lump with Ultrasound (US) scan revealed a cystic lesion in the left side neck favouring a BC. A left sided solitary thyroid nodule with thyroiditis was also incidentally reported. Fine needle aspiration cytology (FNAC) of the cystic fluid showed cytological features of a benign cystic lesion. Further radiological assessment with contrast enhanced CT scan revealed the presence of 3.2cm x 2.2cm septated cystic lesion in left submandibular region with calcific foci medially, compatible with a BC (Fig 2 and 3).



Figure 2. Cystic lesion in the left lateral neck in a Coronal section CT with contrast infusion.

Complete surgical excision of the cyst with its track was done under general anaesthesia and the histological examination reported PTC metastatic to or arising from the BC (Fig 4).

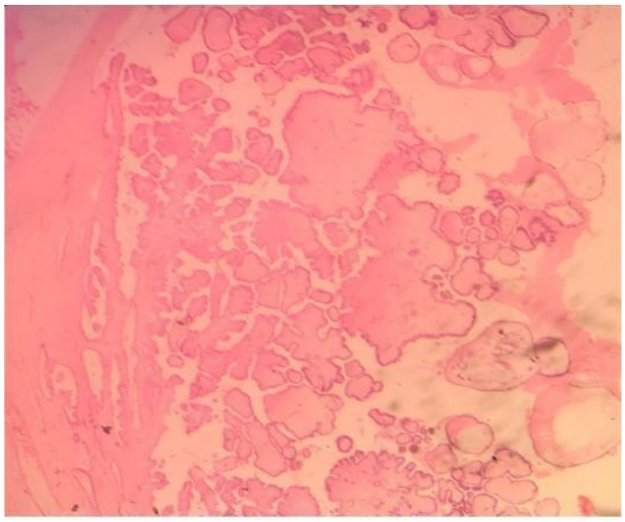


Figure 4. Branchial cyst wall with papillary thyroid carcinoma growth (H and E)

Immune-histochemical analysis of the specimen revealed the neoplastic cells to be staining positive for Thyroid Transcription Factor (TTF-1).

US guided FNAC of the solitary thyroid nodule revealed suspicious lesion (Bethesda Thy4). Thyroid functional tests were in normal range. Total thyroidectomy and ipsilateral selective neck dissection was subsequently performed and the growth of an unencapsulated classical PTC in thyroid gland was confirmed by histology. The patient was then treated with radioactive iodine therapy at the oncology clinic and is disease free for 2 years in follow up.

DISCUSSION

BC are embryological remnants in the neck that are lined by squamous epithelium with frequent presence of lymphoid tissue within[1]. The aetiology of BC is not universally agreed upon. The most accepted theory is the incomplete involution of embryonic pharyngeal clefts, thus the name BC. However, recent theories suggest the formation of BC to cystic degeneration of cervical lymph node triggered by epithelial inclusions from aero- digestive tract and glandular tissue [6,7]. While BC often remain asymptomatic, it can present as a swelling in the lateral neck, usually secondary to filling of the potential cystic space as a result of inflammation [1,2]. Surgical excision remains the standard treatment option for the management of BC [2]. However newer studies show sclerotherapy to be safe and effective in certain cases [8]. Rarely, the cystic mass could also harbour neoplastic tissue metastasizing from elsewhere, with squamous cell carcinoma being the commonest malignancy to be found in it [4]. A retrospective review in a single institution found that out of twenty-eight patients who underwent excision of branchial cysts,[3] was subsequently found to have malignant tissue in it [9]. BC are usually diagnosed in the first decade of life, but presentation in adults is not uncommon. When presenting later in life, the possibility of malignancy should always be considered at the top of the differential [2]. Other probable causes presenting as lateral cystic neck

masses should also be kept in mind even though the diagnosis of BC is made pre-operatively. In a 10-year retrospective study, 14 out 99 patients who underwent surgery for BC were found to have other causes which were missed during the initial assessments [2]. Some of these were cysts with caseous necrosis, metastatic node with cystic changes, cysts of submandibular gland, Warthin tumour, etc.

The presence of PTC in BC is rare in literature and seldom diagnosed pre-operatively. US scan and FNAC remain the first line of work-up in neck masses. Presence of solid components, dystrophic calcifications, being ill-defined and thick walled in US scan raises alarm for the possibility of the cyst to contain neoplastic tissue [10,11]. Thyroglobulin levels in the fine needle aspirate can be a better diagnostic tool than cytology alone in suspected PTC metastasis[12]. But the use of which is unwarranted except in the case of established thyroid malignancy. Use of thyroid functional tests is extremely limited due to majority of patients with PTC having normal thyroid function. It is suggested that the US imaging is to be accompanied by either computed tomography (CT) or magnetic resonance imaging (MRI), especially in older population presenting with cystic mass in lateral neck [9]. Presence of septations, heterogeneous attenuation and extracapsular spread are features suggestive of malignancy in CT [9].

Differentiating PTC that has metastasized to the BC from PTC arising from ectopic thyroid tissue remnant of BC can be challenging [13,14]. Features confirming the latter are the presence of normal ectopic thyroid tissue within the cyst and the exclusion of PTC in the thyroid gland after meticulous histological examination [5]. Immuno-histochemical markers for thyroid tissue such as thyroglobulin and TTF-1 are useful in revealing the origin of undifferentiated tissue to thyroid tissue but fail to distinguish between the normal thyroid and ectopic thyroid tissues found elsewhere. Thus, primary PTC arising from BC are extremely rare and are limited to few case reports[6,10,13,14].

Once the presence of PTC is found in the BC, a total thyroidectomy is recommended and selective neck dissection should be considered even in the absence of suspicious nodules by US scan and FNAC[4,6]. Adjuvant radioactive iodine is effective in the case of residual thyroid tissue or malignancy is suspected[6].

Despite frequently having regional metastasis at the initial presentation, PTC has better prognosis compared to other thyroid malignancies. Overall survival for 10 years can reach as high as 90% if the patient is below young and the treatment is commenced early[10].

CONCLUSION

BC are common congenital anomalies with contentious theories of aetiology. BC harbouring malignancies are rare, but caution should be taken about that possibility especially when presented later in life. Although pre-operative examinations and investigations may aid in the management, diagnosis of PTC in BC may only be possible through histological examination post-operatively. Diagnosis of primary PTC should be reserved only to cases in which a primary tumour is excluded from the thyroid gland histologically.

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Ethical approval and consent to participate

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Conflicts of interest

The authors declare no conflict of interest.

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RESEARCH ARTICLE

EVALUATION OF SALIVARY VITAMIN D3 LEVELS AND ITS ROLE IN THE PATHOGENESIS OF ORAL SUBMUCOUS FIBROSIS- A CASE CONTROL STUDY

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ABSTRACT

Background: Oral Submucous Fibrosis (OSMF) is a chronic, progressive disorder characterized by fibrosis of the oral mucosa, leading to restricted mouth opening and significant functional impairment. It is strongly associated with areca nut consumption and is classified as a potentially malignant disorder. Vitamin D, known for its pleiotropic effects, has been implicated in modulating fibrosis, inflammation, and oxidative stress, which are key factors in OSMF pathogenesis.

Objective: The aim of the study was to evaluate the salivary levels of 25(OH)D3 in patients with OSMF and explore a potential correlation between vitamin D3 deficiency and the progression of OSMF.

Methods: A comparative analysis was conducted to assess salivary Vitamin D levels in OSMF patients and healthy controls. The potential mechanisms by which Vitamin D influences collagen metabolism, immune responses, and oxidative stress were reviewed in the context of existing literature.

Results: The study revealed significantly lower salivary Vitamin D levels in OSMF patients (mean: 36.39 ng/ml) compared to healthy controls (mean: 54.01 ng/ml), with a statistically significant difference (p-value = 0.010). Vitamin D deficiency was associated with increased fibrosis due to dysregulated fibroblast activity, impaired matrix metalloproteinase (MMP) expression, and upregulated transforming growth factor-beta (TGF-β). Additionally, low Vitamin D levels correlated with enhanced inflammation, oxidative stress, and a higher potential for malignant transformation.

Conclusion: The findings highlighted a strong association between Vitamin D deficiency and OSMF pathogenesis. Given its antifibrotic, anti-inflammatory, and antioxidant properties, Vitamin D supplementation may serve as a potential adjunctive therapy for OSMF management. Future research should explore the therapeutic benefits of Vitamin D in preventing disease progression and reducing the risk of malignant transformation.

Keywords: Vitamin D, OSMF, fibrosis, collagen

INTRODUCTION

Oral Submucous Fibrosis (OSMF) is a chronic, progressive, and potentially debilitating disorder that predominantly affects the oral cavity and, in advanced stages, may involve the pharynx and upper esophagus. It is widely recognized as a potentially malignant condition and a collagen metabolic disorder, marked by excessive deposition of collagen fibers, which leads to mucosal rigidity, blanching, burning sensation, and restricted mouth opening.[1]

Over time, the affected tissues lose elasticity and resilience, resulting in functional impairment of the oral structures. Severe cases may present with additional complications such as tongue and soft palate hypomobility, xerostomia, gustatory deficits, pharyngeal and esophageal fibrosis, hearing disturbances, sunken cheeks, muscular atrophy, hoarseness of voice, and nasal speech, significantly impairing the patient's quality of life.[1]

The pathogenesis of OSMF is multifactorial and involves a disruption in collagen homeostasis, characterized by upregulated collagen synthesis and downregulated degradation.[2]

Several risk factors have been identified, among which the most prominent are the use of areca nut, tobacco products, and betel quid—substances widely consumed across South and Southeast Asia. Epidemiological studies conducted in India report that individuals with mixed habits (such as chewing areca nut, gutkha, smoking, and consuming tobacco in various forms) are at a particularly high risk of developing OSMF.[3] The condition affects individuals across a broad age spectrum (11–60 years), with a higher prevalence observed in males (0.2–2.3%) compared to females (1.2–4.6%).[3 4]

The ready availability and cultural acceptance of commercial smokeless tobacco products, such as gutkha, have been closely linked to the rising incidence of OSMF.[4 5] India currently bears the highest global burden of oral cancer, with an estimated 75,000–80,000 new cases reported annually.[5]

Gutkha, in particular, has emerged as a major contributing factor not only in the onset of OSMF but also in its malignant transformation.[6]

It contains several cytotoxic substances, including free radicals like hydroxyl radicals (OH), which are capable of causing DNA damage and initiating carcinogenesis.[7]

These reactive species disrupt the normal cellular environment and promote the transition of OSMF to oral squamous cell carcinoma (OSCC). This highlights the need for vigilant screening and early diagnosis of OSMF to intercept malignant progression.

The carcinogenic progression of OSMF follows a multistep model, transitioning from chronic inflammation and fibrosis to epithelial dysplasia and ultimately invasive carcinoma.[4 5] This model underscores the urgent need to identify early biomarkers capable of detecting premalignant changes before the onset of overt malignancy. Translational oncology is increasingly focusing on molecular approaches to aid early diagnosis and improve therapeutic outcomes.[6 7]

A critical mechanism implicated in carcinogenesis is the evasion of apoptosis—a process essential for maintaining tissue homeostasis. Resistance to apoptosis is often associated with therapy resistance and tumor recurrence in OSCC.[8] Consequently, agents that can restore apoptotic pathways or modulate the tumor microenvironment have become the focus of ongoing research.

Recent studies have pointed to the pleiotropic role of Vitamin D in regulating fibrosis, inflammation, immunity, and cellular proliferation.[9] Beyond its classical role in calcium homeostasis, Vitamin D—particularly its active form, 1,25-dihydroxyvitamin D3—has shown potential in modulating extracellular matrix remodeling, inhibiting fibrogenic pathways, and regulating immune responses.[9] It has been found to downregulate pro-fibrotic cytokines such as transforming growth factor-beta (TGF-β), enhance the expression of matrix metalloproteinases (MMPs), and suppress fibroblast activation—all of which may be beneficial in halting or reversing the fibrotic changes characteristic of OSMF.[9] Additionally, Vitamin D exerts anticancer effects by inhibiting cell proliferation, angiogenesis, and metastasis while promoting differentiation and apoptosis in various cell types, including oral keratinocytes.

Despite these promising biological roles, there is a significant knowledge gap regarding the relevance of Vitamin D status—particularly Vitamin D3 levels—in patients with OSMF. While serum Vitamin D assessments are common in systemic

diseases, the use of saliva as a diagnostic fluid has gained traction due to its non-invasive, cost-effective, and convenient nature. Salivary diagnostics offer a viable alternative for early disease detection, especially in conditions localized to the oral cavity. However, studies investigating salivary Vitamin D3 levels in OSMF patients are less, and its potential as a biomarker for disease presence, severity, or progression remains underexplored.

Given the complex interplay between fibrosis, oxidative stress, and carcinogenesis in OSMF, and the multifaceted role of Vitamin D in modulating these pathways, this study seeks to bridge the existing research gap. The primary aim is to evaluate and compare the salivary Vitamin D3 levels in individuals diagnosed with OSMF and healthy controls, to investigate whether a significant correlation exists between Vitamin D3 status and OSMF pathogenesis. Furthermore, by identifying any potential deficiency or alteration in salivary Vitamin D3 levels, this study aims to assess the feasibility of using it as a non-invasive diagnostic biomarker and explore its therapeutic implications in the management and prevention of malignant transformation in OSMF.

MATERIALS AND METHODS

A prospective cohort study was conducted at Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India, involving 40 participants. The study protocol received approval from the Scientific Review Board (IHEC/SDC/UG-2294/24/PHARM/095). Participants were aged between 25 and 70 years.

The study included two groups: the case group comprised 20 patients histopathologically diagnosed with Oral Submucous Fibrosis (OSMF), while the control group consisted of 20 healthy individuals without systemic disorders. Exclusion criteria included individuals taking Vitamin D supplements, those undergoing chemotherapy, radiotherapy, or any surgical procedures (except biopsy), and patients with systemic conditions known to influence salivary Vitamin D levels, such as parathyroid disorders, rickets, osteomalacia, and sarcoidosis. Additionally, individuals with active infections (e.g., hepatitis, HIV, tuberculosis), chronic kidney or liver disease, malnutrition, or metastasis were excluded.

Medical histories were recorded, clinical examinations were performed, and informed consent was obtained from all participants. Unstimulated saliva samples (2 ml) were collected between January and July 2024 from each subject between 8:00 a.m. and 9:00 a.m. Participants were instructed to follow their routine South Indian diet the night before and on the morning of sample collection. Samples were centrifuged at 5000 rpm for 10 minutes and stored at 4°C for further analysis.

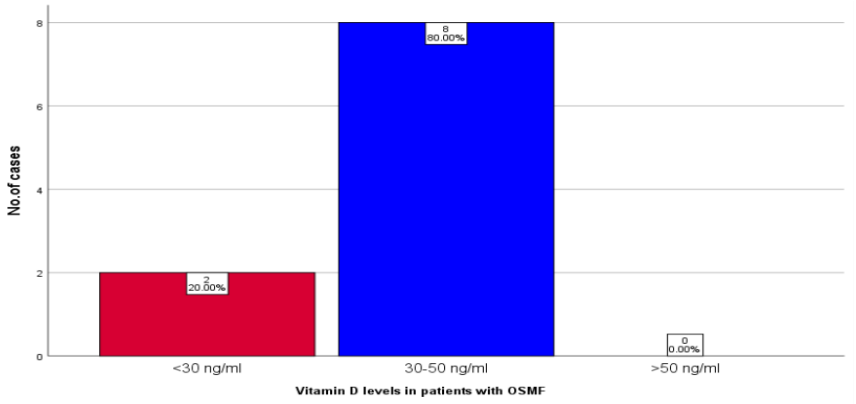
Vitamin D levels (total 25-hydroxy vitamin D) were measured using the Vitamin D Quanti Microlisa kit, which employs a delayed competitive ELISA method. Salivary and serum Vitamin D levels were compared between the case and control groups. To eliminate dietary influences, a diet history was documented, and individuals consuming Vitamin D-fortified foods or supplements were excluded.

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 23.0 (Armonk, NY: IBM Corp.). A paired t-test was used to assess correlations between variables, with a p-value of ≤ 0.05 considered statistically significant.

RESULTS

Expression of salivary 1-25dihydroxycholecalciferol levels in patients with Oral Submucous Fibrosis

Salivary1,25dihydroxycholecalciferol levels in patients undergoing orthodontic treatment was evaluated using delayed competitive ELISA. There was a mean salivary1-25dihydroxycholecalciferol level of **36.39 ng/ml** in patients with Oral Submucous Fibrosis. (Fig. 1)



Figur.1 Graph showing the mean salivary 1-25 dihydroxycholecalciferol levels in patients with OSMF

Expression of salivary 1-25dihydroxycholecalciferol levels in healthy subjects

Salivary1,25dihydroxycholecalciferol levels in patients undergoing orthodontic treatment was evaluated using delayed competitive ELISA. There was a mean salivary1-25dihydroxycholecalciferol level of **54.01 ng/ml** in healthy subjects (Figure 2).

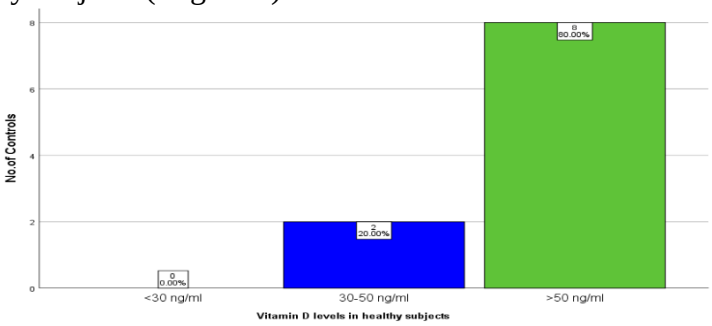


Fig.2 Graph showing the mean salivary 1-25 dihydroxycholecalciferol levels in normal healthy subjects

Comparison of salivary 1-25dihydroxycholecalciferol levels in patients with Oral Submucous Fibrosis and in healthy subjects

In order to compare salivary levels of vitamin D in two groups, paired t-test was used. The results showed that there was a significant difference between the levels of salivary vitamin D in healthy subjects (mean 54.01ng/dl) and salivary vitamin D levels in people with oral submucous fibrosis (mean 36.39 ng/dl) (p-value = 0.010). The results clearly indicate that the level of salivary vitamin D in patients with OSCC was less than that of healthy subjects.(Table).

Table 1. Table showing the comparison of salivary vitamin D3 levels between the two groups done using paired- t-test.

Paired t test		
STUDY GROUP	**Mean**	**Significance (2 Tailed)**
CASE (Patients with OSMF)	36.39 ng/dl	
CONTROL (Healthy subjects)	54.01 ng/dl	0.010

DISCUSSION

Oral Submucous Fibrosis (OSMF) is a progressive, chronic condition that primarily affects the oral cavity and, in some cases, extends into the oropharynx and esophagus. This disease manifests insidiously, characterized by a gradual loss of oral mucosal elasticity, increasing stiffness, and limited mouth opening, often resulting in impaired speech, difficulty chewing, and swallowing challenges. As fibrosis progresses, it deeply compromises a patient's quality of life. The fundamental pathological process driving OSMF is excessive collagen deposition—resulting from both overproduction and inadequate degradation of extracellular matrix components, particularly collagen. This disruption in collagen homeostasis underscores the irreversible nature of the disease in many advanced cases, despite ongoing research into potential therapeutic targets and interventions.[1]

Among the various etiological agents implicated, areca nut chewing remains the primary contributor, frequently compounded by the use of betel quid and gutkha. These habits are particularly widespread in South and Southeast Asia. The compounds present in areca nut—such as alkaloids (arecoline), tannins, and reactive oxygen species (ROS)—stimulate fibroblasts and enhance collagen synthesis while suppressing the enzymatic systems responsible for its degradation. Over time, the cumulative exposure to these irritants induces a sustained inflammatory response and promotes fibrosis. Alarmingly, OSMF is classified as a potentially malignant disorder, with studies suggesting a 7% to 13% risk of transformation into oral squamous cell carcinoma (OSCC).[10 11]

This malignant potential, coupled with the irreversible fibrotic changes in advanced cases, calls for a more refined understanding of its pathogenesis and alternative therapeutic strategies. One such promising area of exploration lies in Vitamin D, a fat-soluble secosteroid traditionally known for its role in calcium homeostasis and bone metabolism. More recently, however, its pleiotropic effects—including anti-inflammatory, antifibrotic, antioxidant, and anticancer properties—have attracted attention across multiple fields of research. Vitamin D exists in two primary forms: D_2 (ergocalciferol) and D_3 (cholecalciferol), the latter of which is synthesized in the skin upon exposure to ultraviolet B (UVB) light and can also be obtained through dietary sources. In the body, it undergoes hydroxylation in the liver to form 25-hydroxyvitamin D [25(OH)D], the major circulating form, and is further activated in the kidneys to 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], which binds to the Vitamin D receptor (VDR) expressed in a variety of tissues—including the oral mucosa.

The activation of VDRs initiates a cascade of gene transcription events that regulate several physiological processes. In the context of OSMF, the most relevant mechanisms include modulation of fibroblast behavior, regulation of matrix metalloproteinases (MMPs), inhibition of pro-fibrotic signaling, and attenuation of chronic inflammation. The pathological hallmark of OSMF—excessive collagen accumulation—is influenced by fibroblast hyperactivity. Interestingly, $1,25(OH)_2D$ has been shown to reduce fibroblast proliferation and downregulate collagen gene expression, thus curbing the primary pathological mechanism of the disease. Moreover, Vitamin D plays a key role in balancing the enzymes that remodel the extracellular matrix. It enhances the expression of MMPs, particularly MMP-1 and MMP-9, while simultaneously suppressing tissue inhibitors of metalloproteinases (TIMPs), which are known to inhibit collagen breakdown. This dual action facilitates the degradation of excess collagen and could potentially slow down or reverse the fibrotic changes seen in early-stage OSMF. The Transforming Growth Factor-Beta (TGF-β) pathway, a well-established mediator of fibrosis, is also a significant target of Vitamin D action. TGF-β promotes fibroblast activation and encourages cross-linking of collagen fibers, which results in the stiff, inelastic mucosa characteristic of OSMF. Vitamin D has been reported to suppress this pathway, thereby impeding the perpetuation of fibrosis.[12–15]

These mechanisms are not unique to the oral cavity. In fact, similar antifibrotic effects of Vitamin D have been documented in other chronic fibrotic conditions, including hepatic fibrosis and idiopathic pulmonary fibrosis. It seems likely that these shared pathways of fibrogenesis could be therapeutically modulated across various tissues through Vitamin D-based interventions.

Another critical factor in OSMF is persistent inflammation, which acts both as a trigger and a driver of disease progression. The habitual use of areca nut and tobacco contributes to sustained mucosal irritation and generates a pro-inflammatory environment marked by elevated levels of cytokines such as IL-6, TNF-α, and IFN-γ. These molecules not only amplify local inflammation but also promote fibroblast activation. Here too, Vitamin D exerts a protective role. By downregulating pro-inflammatory cytokines and enhancing anti-inflammatory mediators like IL-10, Vitamin D helps re-establish immune equilibrium. Inhibiting the nuclear factor-kappa B (NF-κB) signaling pathway—another key driver of chronic inflammation and fibrosis—further strengthens this immunomodulatory profile. These anti-inflammatory effects have been supported by findings from researchers like Kongsbak et al. (2013) and Prietl et al. (2013), who have illustrated the broad-spectrum immune-regulatory capabilities of Vitamin D.[17] [18]

Yet, inflammation is not the only pathological factor exacerbated by lifestyle habits associated with OSMF. Oxidative stress, largely fueled by the ingestion of free radicals in areca nut and tobacco, contributes significantly to tissue damage. Reactive oxygen species (ROS) initiate lipid peroxidation, damage DNA, and denature proteins, setting the stage for fibrosis and potentially malignancy. Vitamin D, as it turns out, possesses potent antioxidant properties. It promotes the expression of endogenous antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPx), which collectively neutralize ROS and protect against cellular injury. Furthermore, by reducing lipid peroxidation, Vitamin D decreases the formation of harmful metabolites that perpetuate tissue damage and inflammatory responses.

The malignant transformation potential of OSMF further highlights the necessity of interventions that can act at multiple levels of disease progression. Remarkably, Vitamin D also demonstrates anticancer properties. It inhibits angiogenesis—disrupting the vascular supply essential for tumor growth—and promotes apoptosis in neoplastic cells. By regulating keratinocyte proliferation and reducing epithelial-to-mesenchymal transition (EMT), Vitamin D supports cellular differentiation and reduces the | risk of dysplasia and invasive transformation.

Garland et al. (2006) emphasized the protective role of Vitamin D against a variety of cancers, lending further weight to its potential relevance in managing premalignant oral disorders like OSMF.[18–20]

In the present study, we observed that OSMF patients exhibited significantly lower salivary Vitamin D3 levels compared to healthy individuals. The average level in affected subjects was 36.39 ng/ml, notably lower than the 54.01 ng/ml recorded in controls. This difference was statistically significant ($p = 0.010$), suggesting a meaningful association between Vitamin D deficiency and the pathogenesis of OSMF. While causation cannot be definitively established from a cross-sectional study, the data support the hypothesis that insufficient Vitamin D may worsen fibrosis, impair mucosal healing, and weaken immune surveillance. In a condition known for its resistance to treatment and potential for malignancy, such findings should not be taken lightly.

Nonetheless, this study is not without limitations. Salivary Vitamin D levels may be influenced by various factors including hydration status, salivary flow rate, and individual metabolic variations. Future studies with larger sample sizes and serum Vitamin D evaluations could help corroborate these results and clarify the pathophysiological links further.

The implications of this study are promising. Ensuring adequate Vitamin D levels through supplementation or lifestyle changes may offer a low-cost, low-risk adjunctive strategy for managing OSMF. Therapeutic possibilities include: (i) Vitamin D Supplementation—either oral or injectable forms to address deficiency and potentially reverse early fibrotic changes; (ii) Dietary Interventions—incorporating foods rich in Vitamin D such as oily fish, fortified dairy products, and egg yolks; (iii) Sunlight Exposure—sensible UVB exposure can significantly enhance endogenous Vitamin D synthesis; (iv) Behavioral Modifications—cessation of areca nut and tobacco use remains critical and may work synergistically with Vitamin D-based interventions; and (v) Adjunctive Therapy—integrating Vitamin D with current modalities such as antioxidants, intralesional corticosteroids, and enzymes like hyaluronidase to improve

therapeutic response.[21 22]

While OSMF remains a complex disorder with multifactorial etiology, Vitamin D appears to intersect with several key aspects of its pathogenesis—fibrosis, inflammation, oxidative stress, and malignant transformation. It is not a cure, nor a standalone solution, but it does represent a biologically plausible and clinically feasible component of a broader management plan. Future longitudinal and interventional studies are warranted to validate these findings and optimize treatment protocols.

CONCLUSION

There was a significant association between reduced Vitamin D levels and OSMF, reinforcing its role in fibrosis, inflammation, and oxidative stress. The study also showed a decrease in salivary Vitamin D levels of OSMF patients, highlighting the potential of Vitamin D supplementation as an adjunctive strategy to mitigate disease progression and reduce the risk of malignant transformation.

DECLARATIONS

Conflict of interest: No conflict of interest is declared by all the authors.

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Data availability: All data and materials of the study are available and can be provided on request. The corresponding author can be contacted anytime to get the data of the study.

Ethics approval and consent to participate: Ethical Committee Clearance Number: (IHEC/SDC/UG-2294/24/PHARM/095). "The study was approved by the institutional human ethical committee board of Saveetha Dental College and Hospitals. (IHEC/SDC/UG-2294/24/PHARM/095). The protocol of the study was approved by the Scientific Review Board (IHEC/SDC/UG-2294/24/PHARM/095) and it conforms to the provisions of the declaration of Helsinki. Informed consent was obtained from all the patients and their legal guardians by informing and clearly explaining the details of the study. All the methods in the study were performed in accordance with the relevant regulations and guidelines.

Consent to participate: Informed consent was obtained from all the patients and their legal guardians by informing and clearly explaining the details of the study.

Consent for publication: Informed consent was obtained from all the patients and their legal guardians by informing and clearly explaining the details of the study.

Author contributions: KK and SS wrote the manuscript. PR and GJ reviewed the manuscript.

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ORIGINAL RESEARCH

SALIVARY VITAMIN D3 AS A NON-INVASIVE BIOMARKER IN RECURRENT APHTHOUS STOMATITIS- A CASE- CONTROL STUDY

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ABSTRACT

Background: Recurrent aphthous stomatitis (RAS) is a common, painful oral ulcerative disorder with a multifactorial etiology involving immune dysregulation and nutritional deficiencies. Vitamin D, known for its immunomodulatory and anti-inflammatory effects, has been increasingly studied for its role in RAS. While serum vitamin D levels have been explored, salivary vitamin D3 measurement offers a non-invasive alternative.

Objective: To evaluate salivary vitamin D3 levels in patients with RAS compared to healthy controls and assess its potential as a biomarker in disease pathogenesis.

Methods: A case-control study was conducted with 24 patients (12 clinically diagnosed RAS patients and 12 matched healthy individuals). Salivary samples were analyzed for vitamin D3 concentration using delayed competitive ELISA. A paired t test was done used using SPSS software for statistical analysis.

Results: RAS patients showed significantly lower salivary vitamin D3 concentrations (40.96–55 ng/mL) than controls (51.05–60.91 ng/mL), suggesting an association between vitamin D deficiency and RAS ($p < 0.05$). These results support vitamin D's role in modulating immune responses linked to ulcer formation. Saliva proved to be a reliable, non-invasive medium for vitamin D3 assessment.

Conclusion:The findings revealed vitamin D deficiency in patients with RAS and highlight salivary vitamin D3 as a practical biomarker. Incorporating vitamin D screening in clinical evaluation of RAS patients may improve diagnosis and management. Further research, including larger, multicentric studies with genetic and environmental considerations, is needed to clarify causality and optimize vitamin D-based therapeutic strategies.

Keywords: Recurrent aphthous stomatitis, Vitamin D3, Salivary biomarkers, Immune modulation, Oral ulcers

1. INTRODUCTION

Recurrent Aphthous Stomatitis (RAS), commonly referred to as canker sores, is one of the most prevalent ulcerative conditions affecting the oral mucosa. It is characterized by recurrent episodes of painful ulcerations primarily localized to the non-keratinized mucosal surfaces of the mouth, including the inner aspects of the lips, cheeks, floor of the mouth, ventral surface of the tongue, and soft palate. These lesions typically present as small, round or oval ulcers with a sharply demarcated erythematous halo surrounding a yellowish-gray pseudomembranous base. Patients frequently experience prodromal symptoms such as burning, itching, or tingling sensations 1 to 2 days prior to ulcer appearance, which can significantly impair daily functions including eating, speaking, and swallowing. Although RAS predominantly affects younger populations—children, adolescents, and young adults—it can occur at any age and is often characterized by its chronic relapsing nature. Involvement of keratinized tissues like the gingiva and hard palate is less common but has been documented in certain cases, sometimes complicating differential diagnosis with other mucosal disorders [1, 2.] .

The global prevalence of RAS varies widely, ranging from approximately 5% to 25% in different populations, with some studies reporting higher frequencies in females and individuals with a family history, suggesting a multifactorial and possibly hereditary component. Despite decades of research, the precise etiology and pathogenesis of RAS remain incompletely understood, primarily due to its complex interplay of multiple contributing factors. Trauma, whether mechanical or chemical, remains one of the most frequently reported local triggers, often precipitating ulcer formation at sites of mucosal injury. Infectious agents such as Helicobacter pylori and herpes simplex virus have also been proposed as potential microbial triggers, although their definitive role remains controversial [3].

Nutritional deficiencies, particularly in essential vitamins and minerals such as folate, vitamin B12, iron, and zinc, have been consistently implicated in RAS pathophysiology. Deficiencies in these nutrients may impair mucosal integrity, reduce immune competence, and disrupt cellular metabolism, thereby facilitating ulcer formation and delayed healing. Psychological factors including stress and anxiety have been identified as exacerbating agents, potentially through modulation of systemic inflammatory responses and neuroimmunological pathways[4]. Among these factors, immune dysregulation has garnered significant attention, given the clear evidence of altered cellular and humoral immune responses in RAS patients. Studies have demonstrated aberrations in T-cell subsets, increased activation of cytotoxic CD8+ lymphocytes, and elevated pro-inflammatory cytokines such as interleukin (IL)-2, IL-6, and tumor necrosis factor-alpha (TNF-α), pointing towards a localized immune-mediated mucosal injury [5–7].

Vitamin D, a fat-soluble secosteroid hormone traditionally associated with bone metabolism and calcium homeostasis, has emerged as a pivotal modulator of immune function and inflammation in recent years. Synthesized in the skin upon exposure to ultraviolet B radiation and obtained from dietary sources, vitamin D undergoes hydroxylation in the liver and kidneys to form its biologically active form, 1,25-dihydroxyvitamin D3 (calcitriol). Beyond its classical endocrine functions, vitamin D exerts autocrine and paracrine effects on various immune cells by binding to the vitamin D receptor (VDR), a nuclear transcription factor expressed on macrophages, dendritic cells, B and T lymphocytes. These interactions regulate gene expression involved in cytokine production, cell proliferation, and immune tolerance, making vitamin D a crucial factor in maintaining immune homeostasis and preventing excessive inflammatory responses [8, 9.]

Emerging epidemiological and experimental evidence links vitamin D deficiency to an increased risk of several autoimmune and inflammatory diseases such as rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, autoimmune thyroid disorders, and inflammatory bowel disease. Vitamin D influences the immune system by enhancing innate immunity via upregulation of antimicrobial peptides like cathelicidin and defensins, while concurrently suppressing the adaptive immune system by inhibiting dendritic cell maturation and promoting the development of regulatory T cells (Tregs), which maintain peripheral tolerance and prevent autoimmunity. Its ability to downregulate pro-inflammatory cytokines such as IL-17, IL-6, and TNF-α further supports its anti-inflammatory role, making vitamin D deficiency a plausible risk factor or disease modifier in conditions involving immune dysregulation [10–12].

Given the immune-mediated nature of RAS, it is reasonable to hypothesize that insufficient vitamin D levels may contribute to the development, severity, or recurrence of aphthous ulcers. However, current literature examining the relationship between vitamin D status and RAS remains limited and somewhat inconclusive, with most studies relying on serum measurements. Saliva, an easily accessible and non-invasive biological fluid, has recently gained attention for its diagnostic potential in reflecting systemic health conditions. Saliva contains a range of biomolecules including hormones, antibodies, enzymes, and cytokines that mirror serum constituents, allowing for real-time monitoring of physiological and pathological states without the discomfort or risk associated with venipuncture [13].

Recent studies have demonstrated that salivary vitamin D

levels correlate reasonably well with serum concentrations, suggesting saliva as a reliable alternative matrix for assessing vitamin D status, particularly in pediatric, geriatric, or medically compromised populations where blood collection may be challenging. Furthermore, salivary diagnostics offer the potential for repeated measurements, enhancing disease monitoring and management. Despite these advantages, there is a paucity of research focusing on salivary vitamin D levels in oral mucosal diseases such as RAS, leaving a significant knowledge gap regarding the feasibility and clinical relevance of salivary biomarkers in this context [14, 15].

To date, no comprehensive studies have simultaneously assessed and correlated serum and salivary vitamin D levels in patients suffering from RAS. This study aims to address this gap by evaluating these parameters in individuals with recurrent aphthous ulcers compared to healthy controls, thereby elucidating the possible role of vitamin D deficiency in RAS pathogenesis. Moreover, it seeks to explore the potential of salivary vitamin D as a non-invasive biomarker for diagnosing systemic deficiency and monitoring inflammatory status in RAS patients. Understanding this relationship could offer new insights into the immunopathology of RAS and inform more targeted therapeutic interventions, including vitamin D supplementation, which may improve clinical outcomes and quality of life for affected individuals.

2. MATERIAL AND METHODS

The case control study was conducted at a private Dental College and Hospital in Chennai, Tamil Nadu, India. A total of 24 participants were enrolled based on prior statistical sample size estimation using G*Power analysis to ensure adequate study power. The participants were divided into two groups: Group 1 consisted of 12 patients clinically diagnosed with Recurrent Aphthous Stomatitis (RAS), while Group 2 included 12 healthy individuals matched for age and sex, serving as controls. All participants were within the age range of 20 to 40 years.

Ethical approval for the study was obtained from the Institutional Human Ethics Committee. Written informed consent was obtained from all participants prior to enrolment in the study, in accordance with the Declaration of Helsinki.

Each subject underwent a detailed clinical examination by a single trained investigator to reduce inter-examiner variability. The examination recorded data on the size, number, duration, location, recurrence frequency, and morphological characteristics of the ulcers. For consistency and accuracy, all lesions were documented using clinical photographs and standardized assessment criteria.

A comprehensive exclusion protocol was implemented. Individuals with any known systemic illness (e.g., diabetes mellitus, autoimmune disorders), chronic inflammatory diseases, periodontitis, or a history of liver or renal impairment were excluded. In addition, subjects with poor oral hygiene, malnutrition, or those who had taken vitamin D supplements within the last three months were not considered for inclusion. Participants with a history of smoking, alcohol consumption, or ongoing corticosteroid or immunosuppressive therapy were also excluded to eliminate potential confounding variables.

To evaluate dietary influences on vitamin D levels, a structured dietary intake chart was used to record participants' routine food habits, with specific attention to foods rich in vitamin D such as dairy products, egg yolks, fatty fish, and fortified items. This helped to screen for any unintentional dietary supplementation.

Saliva and blood samples were collected under standardized conditions, preferably in the morning hours to account for circadian variations. The samples were stored and processed in accordance with established biosafety and clinical laboratory protocols to measure serum and salivary vitamin D levels using enzyme-linked immunosorbent assay (ELISA). This methodology was designed to ensure accurate comparison between systemic and salivary levels of vitamin D and to assess any correlation with the presence of RAS.

3. RESULTS

The study population included 24 participants, comprising 12 clinically confirmed cases of Recurrent Aphthous Stomatitis (RAS) and 12 healthy, age- and sex-matched controls. Salivary vitamin D_3 levels were quantitatively assessed in all participants using a standardized assay.

Among the RAS group, salivary vitamin D_3 concentrations ranged from 38.96 ng/mL to 55.00 ng/mL, whereas the control group exhibited higher levels, ranging from 51.05 ng/mL to 60.91 ng/mL. The mean salivary vitamin D_3 level was substantially lower in the RAS group compared to the controls.

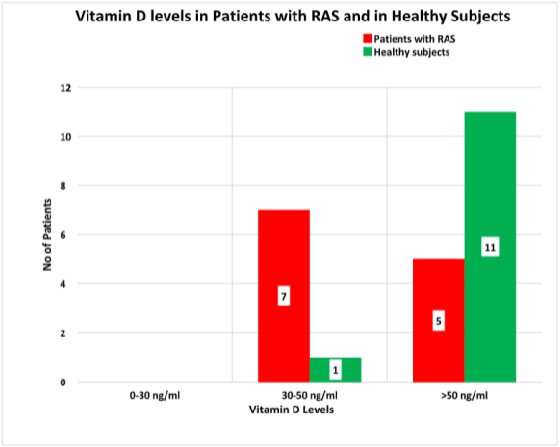


Fig 1 : Graph showing Salivary vitamin D3 levels in patients with RAS and in healthy subjects

A paired t-test was employed to determine the statistical significance of the difference in salivary vitamin D_3 levels between the two groups. The analysis revealed a highly significant difference, with a p-value of 0.001. These findings indicate a statistically and clinically significant reduction in salivary vitamin D_3 concentrations among patients with RAS in comparison to healthy individuals, suggesting a potential association between vitamin D deficiency and the occurrence of aphthous ulcers.(Table 1)

Table.1 Table showing the statistically significant result (p<0.05) Paired-t test done using SPSS software version 23.0

Paired t test		
Study group	**Mean**	**Significance (2-tailed)**
Case group	47.98 ng/ml	0.001
Control group	55.98 ng/ml	

DISCUSSION

Vitamin D, a fat-soluble secosteroid mainly produced in the skin under ultraviolet B exposure, is traditionally recognized for its critical role in calcium and phosphate homeostasis and bone integrity. Yet, over the past decade, its significance has broadened to encompass modulation of immune and inflammatory pathways, raising intriguing possibilities about its involvement in various chronic inflammatory and autoimmune diseases, including recurrent aphthous stomatitis (RAS) [1,16-22]. RAS remains one of the most common oral mucosal disorders, afflicting nearly one-fifth of the population, predominantly adolescents and young adults [23]. Clinically, it manifests as painful, discrete ulcers with erythematous margins and a fibrinous base, preferentially affecting non-keratinized mucosa such as the buccal mucosa and soft palate [24].

Despite decades of research, the precise etiology of RAS remains elusive. Its multifactorial nature is widely accepted, encompassing genetic predispositions, immune dysregulation, microbial factors, hormonal fluctuations, local trauma, psychological stress, and nutritional deficiencies [25]. Among these, vitamin D's role has garnered growing attention, largely due to its immunomodulatory and anti-inflammatory properties that could influence the underlying pathogenic mechanisms of RAS [26]. Unlike classical vitamins, vitamin D acts as a hormone, modulating innate and adaptive immunity, especially through T-cell regulation and cytokine production [27]. The present study's observation of significantly reduced salivary vitamin D3 levels in individuals with RAS compared to healthy controls adds a valuable dimension to this narrative. Earlier investigations predominantly measured serum vitamin D levels, reporting a general trend towards deficiency or insufficiency in RAS patients[28,29]. Few studies have documented significantly lower serum 25(OH)D concentrations in RAS patients versus controls, suggesting systemic hypovitaminosis D may contribute to disease pathogenesis. However, serum sampling, while informative, is invasive and less convenient for repeated monitoring. Saliva, containing hormones, cytokines, antibodies, and enzymes reflective of systemic physiology, has emerged as an attractive alternative for non-invasive diagnostics [30-32]. The present study's use of salivary vitamin D3, quantified by a sensitive delayed competitive ELISA, thus represents a novel, patient-friendly approach, aligning with trends in biomarker research that seek to minimize discomfort and improve clinical applicability [33-36].

Interestingly, the mean salivary vitamin D3 concentration among RAS patients in this study (38.96–55 ng/mL) was consistently lower than in healthy subjects (51.05–60.91 ng/mL). This significant difference supports the hypothesis that insufficient vitamin D levels may skew the immune environment towards a pro-inflammatory state conducive to ulcer formation. This finding is in harmony with recent works, where also reported lower salivary vitamin D3 in RAS patients, further substantiating the utility of saliva as a diagnostic medium [37]. It seems likely that vitamin D insufficiency disrupts the delicate balance of cytokine regulation in oral mucosa. Immunologically, vitamin D's interaction with its receptor (VDR) on immune cells such as macrophages, dendritic cells, and T lymphocytes orchestrates a complex regulatory network. It downregulates pro-inflammatory cytokines including IL-2, IL-6, and TNF-α, while promoting anti-inflammatory cytokines like IL-10, thus maintaining mucosal homeostasis. A deficiency could therefore precipitate exaggerated inflammation and impaired healing characteristic of RAS lesions. These mechanistic insights demonstrated that vitamin D deficiency enhances susceptibility to oral mucosal damage through modulation of T-helper cell responses . Moreover, vitamin D's role in maintaining epithelial barrier integrity and promoting wound healing may be especially pertinent in the recurrent breakdown and slow repair seen in RAS [15].

Vitamin D deficiency has also been implicated in other inflammatory oral diseases, such as periodontitis and oral lichen planus, conditions sharing some immunopathological features with RAS [35-37]. This overlap lends credibility to the hypothesis that vitamin D insufficiency may represent a common denominator in oral mucosal inflammatory diseases. However, unlike periodontitis where bacterial biofilm plays a major role, RAS's etiopathogenesis remains less well-defined, making the identification of modifiable risk factors like vitamin D status particularly valuable [37].

Micronutrient deficiencies in general have been associated with RAS, as evidenced by studies, which

found prevalent deficiencies in B-complex vitamins in recurrent oral ulcer patients and noted clinical improvement with targeted supplementation[38]. Contrastingly, Lalla et al. (2021) reported that broad-spectrum multivitamin supplementation did not significantly reduce ulcer frequency or severity, raising doubts about the effectiveness of generalized supplementation without addressing specific deficiencies[39]. Our findings reinforce the notion that targeted assessment and correction of vitamin D deficiency might offer a more focused and effective therapeutic approach rather than indiscriminate multivitamin use.

Adding complexity, polymorphisms in the VDR gene have been linked to various autoimmune and inflammatory diseases, including RAS[40]. These genetic variations can alter receptor function and vitamin D signaling, influencing individual susceptibility to vitamin D deficiency and disease manifestation. Environmental factors, notably geographic location and cultural practices, further modulate vitamin D levels. Paradoxically, populations in sun-rich regions such as South Asia often exhibit high rates of hypovitaminosis D, due to factors like sun-avoidance behaviors, clothing customs, and indoor lifestyles. This cultural-environmental interplay underscores the multifactorial nature of vitamin D deficiency and its downstream effects in diseases like RAS.

The methodological strengths of this study lie in its non-invasive salivary analysis and the use of delayed competitive ELISA, a robust and specific assay for steroid hormones, which improves reliability over earlier colorimetric methods[41]. Saliva sampling is painless, easily repeatable, and suitable for chairside screening, potentially enabling earlier detection and monitoring of vitamin D status in patients with oral ulcers.

Nonetheless, certain limitations temper the findings. The modest sample size restricts the generalizability of results and calls for larger multicentric studies to validate these observations. Additionally, while salivary vitamin D correlates with serum levels, it may not fully mirror systemic status due to factors such as salivary flow, circadian variation, and oral hygiene practices[42,43]. Moreover, confounders like dietary vitamin D intake, sun exposure habits, skin pigmentation, and seasonal effects were not fully controlled, which could influence vitamin D levels independently of RAS presence.

Furthermore, the absence of genetic profiling for VDR polymorphisms or enzymes involved in vitamin D metabolism constitutes a missed opportunity to dissect gene-environment interactions in this context. Incorporating such analyses in future research could elucidate underlying susceptibilities and refine personalized treatment strategies[24]. It would also be useful to investigate longitudinal changes in salivary vitamin D levels relative to RAS flare-ups and remission phases.

Therapeutically, the observed salivary vitamin D3 deficiency in RAS patients hints at a potentially modifiable risk factor. Pilot intervention studies exploring oral vitamin D supplementation have yielded encouraging results, showing reductions in ulcer frequency and severity, though sample sizes remain small and methodologies heterogeneous. Vitamin D's capacity to enhance mucosal healing and barrier function further supports its therapeutic relevance[34]. Nonetheless, standardized protocols regarding optimal dosage, duration, and formulations are lacking, necessitating rigorously designed randomized controlled trials before routine clinical recommendations can be made.

From a clinical perspective, these findings argue in favor of incorporating vitamin D screening—preferably non-invasive salivary assays alongside serum measures—into the routine diagnostic evaluation of patients with recurrent oral ulcers. Early identification and correction of vitamin D deficiency could reduce disease burden, improve patient quality of life, and potentially prevent progression to more severe mucosal damage. Clinicians should also counsel patients on safe sun exposure practices and encourage dietary sources rich in vitamin D, tailoring advice to individual lifestyles and cultural context.

In essence, this study contributes to a growing body of evidence implicating vitamin D deficiency in the pathogenesis of RAS. While the data are compelling, causality remains to be definitively established. It seems likely that vitamin D insufficiency acts synergistically with genetic, environmental, and immunological factors to promote mucosal vulnerability and ulceration. Further expansive, longitudinal, and mechanistic studies are essential to unravel these complex interactions and to translate findings into effective preventive and therapeutic strategies.

Ultimately, if vitamin D supplementation proves effective, it could offer a safe, low-cost, and accessible adjunct treatment for a condition that, despite being benign, significantly impairs oral comfort and patient wellbeing. Emphasizing patient-centered, individualized care that integrates nutritional, genetic, and lifestyle factors will likely be the key to advancing management of recurrent aphthous stomatitis.

CONCLUSION

This study demonstrates a significant association between lower salivary vitamin D_3 levels and recurrent aphthous stomatitis, suggesting a potential role of vitamin D deficiency in its pathogenesis. These findings support the need for further research on vitamin D supplementation as a preventive or therapeutic approach for managing RAS.

DECLARATIONS

Conflict Of Interest

All the authors declare that there was no conflict of interest in the present study.

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Ethics approval and consent to participate: Ethical Committee Clearance Number: (IHEC/SDC/UG - 2273/24/PHARM/096). "The study was approved by the institutional human ethical committee board of Saveetha Dental College and Hospitals. (IHEC/SDC/UG - 2273/24/PHARM/096). The protocol of the study was approved by the Scientific Review Board (IHEC/SDC/UG - 2273/24/PHARM/096) and it conforms to the provisions of the declaration of Helsinki. Informed consent was obtained from all the patients and their legal guardians by informing and clearly explaining the details of the study. All the methods in the study were performed in accordance with the relevant regulations and guidelines.

Consent to participate: Informed consent was obtained from all the patients and their legal guardians by informing and clearly explaining the details of the study.

Consent for publication: Informed consent was obtained from all the patients and their legal guardians by informing and clearly explaining the details of the study.

Data availability: All data and materials of the study are available and can be provided on request. The corresponding author Dr. Sandra Sagar can be contacted anytime to get the data of the study.

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ORIGINAL ARTICALE

ASSOCIATION BETWEEN SALIVARY VITAMIN D3 LEVELS AND DENTAL CARIES IN CHILDREN: A CASE-CONTROL STUDY

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ABSTRACT

Background: Dental caries remains a significant global oral health concern, particularly among children. Vitamin D3 plays a crucial role in tooth mineralization and immune function, and its deficiency has been associated with increased caries risk. Traditional assessment of Vitamin D3 involves serum measurements, but saliva has recently emerged as a promising non-invasive alternative, especially in pediatric populations.

Aim: This study aimed to assess salivary Vitamin D3 levels in children with dental caries compared to healthy individuals.

Methods:A total of 40 children aged 10–15 years were divided into two groups: 20 children with dental caries (ICDAS II code 5) and 20 healthy controls (ICDAS II code 0). Unstimulated saliva samples were collected between 8–9 a.m., centrifuged, and stored at 4°C. Levels of 1,25-dihydroxycholecalciferol were quantified using an enzyme-linked immunosorbent assay (ELISA). Statistical analysis was performed using a paired t-test.

Results:The mean salivary Vitamin D3 level in the caries group was 30.87 ng/dL, significantly lower than the 40.125 ng/dL observed in healthy controls ($p = 0.010$). This suggests a strong association between Vitamin D3 deficiency and dental caries occurrence.

Conclusion:Children with dental caries exhibited significantly reduced salivary Vitamin D3 levels. Salivary Vitamin D3 measurement offers a practical, non-invasive approach for early identification of caries risk, particularly in young populations. These findings support further research into the diagnostic utility of saliva-based Vitamin D assessment and its potential integration into preventive pediatric dental care.

Keywords: Dental caries, Vitamin D3, salivary biomarkers

1. INTRODUCTION

Dental caries remains one of the oldest known oral diseases, yet it continues to pose a significant threat to oral health across all age groups. It's remarkable, and somewhat troubling, that a disease so well studied still prevails so widely. Defined by the World Health Organization (WHO) as a localized, post-eruptive,

pathological process of external origin that leads to the softening and eventual destruction of the hard dental tissues, caries is far from a simple problem [1]. It is not just about cavities; it's a reflection of broader issues involving biology, behavior, and environment.

At its core, dental caries results from the interaction of acid-producing bacteria with fermentable carbohydrates in the mouth [2, 3]. These bacteria metabolize sugars and release acids that erode the tooth's surface, leading to demineralization and eventual cavity formation. That part is fairly well understood. However, what often escapes casual observation is how this biological process is embedded within larger socio-economic and cultural systems. The increasing prevalence of caries in many regions—despite decades of public health efforts—suggests that microbial activity is only part of the story. Factors such as genetic changes in bacteria, declining oral hygiene practices, and the widespread availability of refined, sugar-rich foods have likely contributed to this growing burden. Interestingly, populations of lower socio-economic status tend to exhibit higher caries rates, which could be due to limited access to dental care, poor nutritional options, or a lack of public health infrastructure [4].

It's worth asking: why do we still struggle to control something that, in theory, is preventable? Public health strategies like water fluoridation, oral hygiene campaigns, and the promotion of fluoride toothpaste have certainly helped reduce caries in some areas, but their success has been uneven [5]. The fact that caries remains one of the most common chronic diseases—especially in children—points to a gap between knowledge and practice. Prevention seems to require more than just information or tools; it demands systemic change.

This leads to an emerging area of interest in the field: the role of systemic health, and in particular, Vitamin D3, in the development and progression of dental caries. Vitamin D3, or cholecalciferol, is widely recognized for its role in regulating calcium and phosphorus metabolism—both essential for the mineralization of bones and teeth [6, 7]. Yet its significance extends beyond mineral balance. Recent insights suggest that Vitamin D3 also supports the immune system and influences the activity of enamel- and dentin-forming cells, including ameloblasts and odontoblasts. These cells, which are crucial during tooth development, express receptors for Vitamin D3, implicating the vitamin in the structural integrity of dental tissues as well as their defense mechanisms [8, 9].

It seems likely, then, that adequate levels of Vitamin D3 during early development could contribute not only to stronger teeth but also to greater resistance against the microbial and acidic challenges that trigger caries. A deficiency, by contrast, might leave teeth more vulnerable. Despite this plausible link, Vitamin D's role in caries prevention hasn't received as much attention in mainstream dentistry as fluoride has. This could be due to the traditional emphasis on local, surface-level interventions rather than systemic ones.

The standard way to measure Vitamin D status is through serum levels of 25-hydroxyvitamin D3[10]. While accurate, this method is invasive, requiring a blood draw, which is particularly problematic when working with children. Understandably, many parents—and children themselves—are hesitant about routine blood testing for something perceived as non-urgent. This hesitancy poses a practical challenge for researchers and clinicians trying to explore Vitamin D status in pediatric populations.

Interestingly, saliva has recently been proposed as a viable, non-invasive alternative for Vitamin D assessment. Though salivary diagnostics are still gaining traction in mainstream clinical settings, several studies have shown that salivary levels of Vitamin D metabolites correlate with serum levels, offering a gentler approach to data collection. Techniques like ELISA (enzyme-linked immunosorbent assay) have made it possible to measure these levels with reasonable sensitivity and specificity, potentially transforming how we screen for nutrient deficiencies in children.

Nonetheless, there are still questions to be addressed. How reliable is salivary Vitamin D measurement across diverse populations? Are the cut-off values for deficiency the same as those in serum? What confounding factors—such as hydration, circadian rhythms, or oral health status—might influence salivary readings? These are not trivial concerns. But the potential benefits of a child-friendly, needle-free test for a vitamin so critical to oral and general health make this area worth investigating further.

Given the concerning rates of dental caries in children and the emerging evidence of Vitamin D's involvement in tooth integrity and immune defense, this study aims to explore the association between salivary Vitamin D3 levels and dental caries. Using an ELISA-based quantification method, it seeks to evaluate whether saliva can serve as a reliable medium for screening Vitamin D status and predicting caries risk. While it may not offer immediate solutions, such research could pave the way for more holistic, child-friendly approaches to oral health—approaches that consider not just brushing and fluoride, but also the role of nutrition, immunity, and systemic wellness.

2. MATERIALS AND METHODS

A case control was conducted among n = 40 patients at a private dental hospital. The sample size calculation was done using G-power analysis by keeping the alpha error probability as 0.05, allocation ratio as 1 and the power of the study (1-beta error probability) as 0.99, the sample size was calculated as n = 40. The protocol of the study was approved by the Scientific Review Board and it conforms to the provisions of the declaration of Helsinki. This study included forty participants, with twenty healthy individuals showing no signs of caries and twenty diagnosed with dental caries. The subjects were between 10 and 15 years old, within the mixed dentition period. Inclusion criteria restricted participants to those without chronic medical conditions. Healthy participants met the ICDAS II code 0 criteria for dental caries, while those with dental caries were classified under ICDAS code 5 (Table 1). Exclusion criteria included malnourished subjects, individuals with systemic diseases, periodontitis, known chronic/ liver diseases, known bone disorders, and those taking Vitamin D3 supplements.

2 ml of unstimulated saliva sample was collected from each of the forty participants after taking a proper diet history from the patients between 8 a.m. and 9 a.m. Patients taking any form of Vitamin D supplementation or Vitamin D fortified foods were excluded from the study. Patients were instructed to have their routine South Indian diet the previous night and morning of sample collection. These collected samples were centrifuged at 5000 RPM for 10 minutes using Eppendorf vials, stored at 4 °C and then analysed for Vitamin D3 levels using an ELISA kit. The assay involved buffering the samples with a Vitamin D buffer, which resisted pH fluctuations and prevented non-specific binding. Afterward, the samples were treated with biotinylated Vitamin D analog specific for human VDR, followed by the addition of ELISA HRP (horseradish peroxidase) to produce color compounds indicating Vitamin D3 presence. The reaction was halted using a TMB (tetramethylbenzidine) solution when the OD values reached around 0.7 units, and the Vitamin D3 levels were quantified using an ELISA plate analyser. (Fig.1)

The collected primary results data were analysed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp. A paired t-test was done to compare the salivary Vitamin D3 levels between the two groups.

Table 1 Table showing the ICDAS II criteria for caries detection on the pit and fissures.

Code	Description
0	There should be no evidence of caries after prolonged air drying (5 seconds). Surfaces with developmental defects (enamel hypoplasia, fluorosis, attrition, abrasion and erosion) and intrinsic and extrinsic stains will be considered as sound.
1 – First visual change in enamel	When seen wet, there is no evidence of any change in color attributable to the carious activity, but after prolonged air drying a carious opacity or discoloration (White or brown lesion) is visible that is not consistent with the clinical appearance of sound enamel.
2 – Distinct visual change in enamel	The tooth must be viewed wet. When wet there is a (a) carious opacity (white spot lesion) and/or (b) brown carious discoloration which is wider than the natural fissure⁄fossa that is not consistent with the clinical appearance of sound enamel.
3 – Localized enamel breakdown due to caries with no visible dentin or underlying shadow	The tooth viewed wet may have a clear carious opacity (white spot lesion) and/or brown carious discoloration which is wider than the natural fissure ⁄fossa that is not consistent with the clinical appearance of sound enamel. Once dried for approximately 5 seconds there is carious loss of tooth structure at the entrance to, or within, the pit or fissure⁄fossa. If in doubt, or to confirm the visual assessment, the CPI probe was used gently across a tooth surface to confirm the presence of a cavity apparently confined to the enamel.
4 – Underlying dark shadow from dentin with or without localized enamel breakdown	This lesion appears as a shadow of discolored dentin visible through an apparently intact enamel surface which may or may not show signs of localized breakdown (loss of continuity of the surface that is not showing the dentin).
5 – Distinct cavity with visible dentin	Cavitation in opaque or discolored enamel exposing the dentin beneath.
6 – Extensive distinct cavity with visible dentin	Obvious loss of tooth structure, the cavity is both deep and wide and dentin is clearly visible on the walls and at the base. An extensive cavity involves at least half of a tooth surface or possibly reaching the pulp.

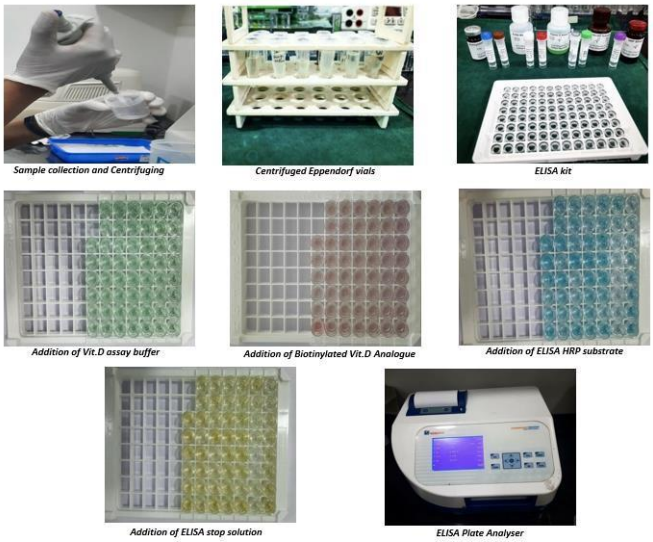


Figure1. Image showing the various steps involved in the evaluation of salivary samples for Vitamin D using ELISA.

RESULTS

Salivary Vitamin D3 levels in patients were analysed using delayed competitive ELISA. There was a mean salivary Vitamin D3 level of 40.125ng/dl in healthy individuals whereas the mean salivary vitamin D3 level of the patients with dental caries was found to be about 30.87 ng/dl. A paired t-test was done to compare the salivary vitamin D3 levels between the two groups. The results revealed a significant difference in salivary Vitamin D3 levels between healthy individuals (mean 40.125 ng/dl) and those with dental caries (mean 30.87 ng/dl), with a p-value of 0.010. (Fig.2) The salivary Vitamin D3 levels in children with dental caries were notably less than those of healthy subjects (Table 2).

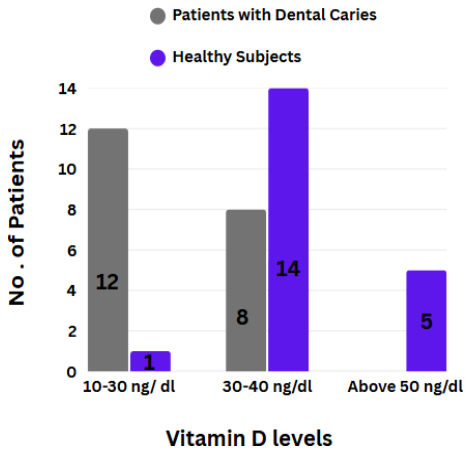


Fig.2 Graph showing Salivary Vit. D levels in patients with dental caries and in healthy subjects.

Table.2 Table showing the statistically significant result (p<0.05) Paired-t test done using SPSS software version 23.0

Paired-t-test		
STUDY GROUP	**MEAN**	**SIGNIFICANCE (2-TAILED)**
Vitamin D levels in patients with dental caries	**30.85 ng/dl**	**0.01**
Vitamin D levels in healthy subjects	**40.125 ng/dl**	

DISCUSSION

Dental caries remains one of the most widespread chronic conditions globally, and it continues to affect individuals of all age groups despite decades of public health efforts. According to WHO estimates, its prevalence ranges from 49% to as high as 83% across various populations [11]. Such staggering numbers point not only to the biological tenacity of the disease but also to the broader socio-environmental conditions that allow it to thrive. In many ways, the modern lifestyle—with its emphasis on processed foods, sugary diets, and often inadequate oral hygiene—has created the perfect storm for dental caries to persist. Although fluoride programs and oral health awareness campaigns have brought measurable improvements in some areas, the disease disproportionately impacts children from disadvantaged socio-economic backgrounds[12, 13]. Early Childhood Caries (ECC), in particular, underscores the urgency of this issue. It does not merely affect teeth; it interferes with a child's speech, eating, sleep, and self-esteem, exerting long-term effects on quality of life.

Vitamin D, long recognized for its importance in bone health, has garnered attention in recent years for its potential role in oral health. The relationship is complex, spanning mineral metabolism, immune modulation, and gene expression. Notably, Vitamin D deficiency has been linked with defective enamel and dentin formation, making teeth more susceptible to demineralization and caries [14, 15]. While Vitamin D2 and D3 are both available in the diet, it is cholecalciferol (Vitamin D3)—primarily synthesized through skin exposure to sunlight—that serves as the biologically active form. This dependency on sun exposure is problematic in regions where cultural practices, climate, or urban lifestyles reduce sunlight exposure, particularly among children. In these contexts, Vitamin D deficiency may be both widespread and underdiagnosed.

The physiological mechanisms by which Vitamin D influences oral health are multifaceted. Severe deficiency,

defined as serum levels below 10 ng/mL, leads to disruptions in calcium and phosphate metabolism. This not only triggers secondary hyperparathyroidism but also alters the functionality of ameloblasts and odontoblasts—the key cells involved in enamel and dentin development [15]. Inadequate Vitamin D signaling in these cells results in hypomineralized tooth structures, which are more prone to decay. These cellular mechanisms are further mediated by the presence of Vitamin D receptors (VDRs), which regulate gene expression critical for dental tissue development [16]. Therefore, Vitamin D's role extends beyond nutrient status and into the realm of molecular regulation of tooth integrity.

Importantly, the effects of Vitamin D deficiency are not confined to the postnatal period. Emerging evidence supports the notion that maternal Vitamin D levels during pregnancy can significantly influence the dental health of the offspring. Several studies have found that inadequate maternal Vitamin D is linked to developmental dental defects in children, particularly when deficiency occurs during the first or second trimester—key periods of odontogenesis [17-19]. This prenatal link highlights how early and subtle disruptions in nutrient status can have structural consequences for primary teeth, often unnoticed until the child begins to show signs of ECC. As such, a life-course approach to Vitamin D sufficiency may be warranted when thinking about oral health promotion.
Beyond its structural roles, Vitamin D is also involved in immunomodulation. Numerous studies advocate for maintaining serum Vitamin D levels above 75 nmol/L to optimize immune responses [20-22]. This becomes particularly relevant in the context of oral health, where the mouth is constantly exposed to a complex and dynamic microbiome. Vitamin D has been shown to upregulate antimicrobial peptides such as cathelicidin and defensins, which are part of the innate immune system and play a protective role in the oral cavity. Therefore, children with adequate Vitamin D levels may be better equipped to mount immune defenses against cariogenic bacteria, adding another layer to its preventive potential.
Meta-analytical evidence further reinforces the association between Vitamin D and dental caries. A landmark review by Hujoel et al. reported that Vitamin D supplementation could reduce caries incidence by as much as 47% [23]. Schroth and colleagues echoed these findings, noting that children with sufficient serum Vitamin D levels consistently had fewer carious lesions [23]. These associations, while not always implying causation, make a compelling case for the protective role of Vitamin D in oral health, particularly among pediatric populations.
Our study builds upon this foundation by demonstrating a statistically significant relationship between lower salivary Vitamin D3 levels and the presence of dental caries in children. While prior investigations have largely relied on serum Vitamin D levels, our findings suggest that salivary assessments can offer comparable diagnostic value. This aligns with reports by Adegboye et al., who found higher caries rates among children with Vitamin D deficiency, and with studies by Hujoel and Schroth that confirmed this inverse relationship between Vitamin D status and caries prevalence [23- 28]. Notably, our study reinforces that these findings hold true even when Vitamin D3 is measured in saliva rather than serum.

The decision to utilize saliva as the diagnostic medium in this study is not merely a methodological choice but also a practical and ethical one. Blood draws in children often pose significant barriers—ranging from fear and discomfort to logistical difficulties in remote or under-resourced settings. Saliva, in contrast, is non-invasive, easy to collect, and does not require specialized equipment or personnel. This makes it an ideal medium for pediatric populations and for use in school-based or community health initiatives. Prior studies, including those by Sari et al., have validated saliva as a feasible and reliable medium for Vitamin D estimation [29-32].

That said, the adoption of salivary diagnostics in clinical practice remains limited. Part of the hesitation stems from a lack of standardized protocols. Although early assays, such as those by Fairney & Saphier and later by Tatsuya Higashi using LC-ESI-MS/MS, have demonstrated promising correlations between serum and salivary Vitamin D levels, broader consensus is lacking [33–38]. Our study contributes to this growing evidence base, affirming that salivary Vitamin D3 assessment—particularly using ELISA techniques—can be both sensitive and practical. As technology advances, it is plausible that saliva-based diagnostics could become standard tools in pediatric oral healthcare.

Nonetheless, no study is without limitations. Ours was conducted with a relatively small cohort, which constrains the generalizability of the findings. The cross-sectional nature of the study also limits causal inference; it is unclear whether low Vitamin D3 precedes caries development or results from it. Seasonal variation—affecting both sun exposure and Vitamin D synthesis—was not rigorously controlled, and this may have influenced salivary Vitamin D levels. Furthermore, while saliva collection offers practical benefits, factors such as time of day, hydration status, and oral hygiene at the time of sampling could impact the concentration of Vitamin D metabolites. These confounding variables warrant attention in future research.

Another consideration is the lack of comprehensive data on confounding lifestyle and genetic factors. For instance,

children's dietary intake, frequency of outdoor activities, skin pigmentation, and even VDR polymorphisms may all modulate the relationship between Vitamin D and caries risk. Future studies should aim to control for or stratify based on these variables to refine our understanding of causality. Longitudinal studies, in particular, could help unravel the directionality of this relationship by tracking children over time as their Vitamin D status and caries risk evolve.

In terms of public health implications, our findings raise important questions about how best to incorporate Vitamin D screening and supplementation into pediatric dental care. If salivary testing can be standardized and validated across diverse populations, it could be integrated into routine dental checkups, much like fluoride varnish or oral hygiene instruction. Such integration would allow early identification of at-risk children, potentially before caries develops. Moreover, targeted interventions—ranging from dietary guidance to supervised sun exposure or supplementation—could be deployed more efficiently.[39-40]

Interestingly, while Vitamin D is often discussed in terms of deficiency, optimal levels may vary between individuals. The conventional thresholds for sufficiency may not reflect ideal levels for dental health, especially when considering the complex interplay of immune response, microbial ecology, and tissue mineralization. Future research might investigate whether personalized Vitamin D targets—based on genetic, environmental, and dietary factors—could yield better preventive outcomes.

This study adds to a growing body of literature that highlights the critical role of Vitamin D3 in oral health, particularly in children. By demonstrating that salivary Vitamin D3 levels correlate with caries status, we offer support for non-invasive, scalable diagnostic strategies that could be transformative for pediatric oral healthcare. While further research is essential to validate these findings and overcome current limitations, the integration of salivary Vitamin D3 screening into preventive dental programs holds considerable promise.

CONCLUSION

Our study reveals a significant association between Vitamin D deficiency and dental caries in children. Given the non-invasive nature of saliva-based assessments, salivary Vitamin D3 levels could serve as a reliable marker for identifying children at risk of developing dental caries. Early detection and Vitamin D supplementation, particularly during prenatal and postnatal periods, may help prevent dental caries during the critical stages of dentition development. Future research should focus on further validating saliva as a diagnostic medium for Vitamin D and exploring its potential in broader public health applications.

DECLARATIONS

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Author contributions

SS and SM wrote the manuscript. GJ and PR reviewed the manuscript.

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Data availability

All data and materials of the study are available and can be provided on request. The corresponding author Dr. Genickson Jeyaraj can be contacted anytime to get the data of the study.

Conflict of interest

No conflict of interest is declared by all the authors.

Ethics approval and consent to participate

Ethical Committee Clearance Number: (IHEC/SDC/FACULTY/23/OPATH/109). “The study was approved by the institutional human ethical committee board of Saveetha Dental College and Hospitals. (IHEC/SDC/FACULTY/23/OPATH/109). The study protocol was approved by the Scientific Review Board (IHEC/SDC/FACULTY/23/OPATH/109) and conforms to the provisions of the declaration of Helsinki. Informed consent was obtained from all the patients and their legal guardians by informing and clearly explaining the details of the study. All the methods in the study were performed in accordance with the relevant regulations and guidelines.

Consent to participate

Informed consent was obtained from all the patients and their legal guardians by informing and clearly explaining the details of the study.

Consent for publication

Informed consent was obtained from all the patients and their legal guardians by informing and clearly explaining the details of the study.

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CLINICAL RESEARCH

COMPARING BUCCAL FAT PAD AND NASOLABIAL FLAP APPROACHES FOR ORAL SUBMUCOUS FIBROSIS RECONSTRUCTION

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ABSTRACT

Objective: This study evaluated and compared the effectiveness of the buccal fat pad and nasolabial flap techniques in surgically treating oral submucous fibrosis, with emphasis on improvements in mouth opening and oral commissure width after surgery.

Materials and Methods: Twenty patients with clinically confirmed oral submucous fibrosis and a mouth opening less than 20 mm were included. They were divided equally into two groups: Group 1 (10 patients) received treatment with a nasolabial flap, while Group 2 (10 patients) was managed using the buccal fat pad. Measurements of incisal mouth opening and oral commissural width were taken before and after surgery. Data analysis was conducted using paired and unpaired t-tests, considering p-values ≤ 0.05 as statistically significant.

Results: Both surgical interventions resulted in significant postoperative increases in mouth opening and commissural width. Notably, patients in the buccal fat pad group exhibited a more pronounced improvement in these parameters at the 6-month postoperative mark compared to those treated with the nasolabial flap.

Conclusion: The nasolabial flap and buccal fat pad both serve as viable surgical methods for managing oral submucous fibrosis. The buccal fat pad demonstrated superior functional outcomes in terms of mouth opening and commissural width, although nasolabial flaps remain an effective treatment alternative.

Keywords: Buccal fat pad, commissural width, mouth opening, nasolabial flap, Oral submucous fibrosis

Keywords: Buccal fat pad, commissural width, mouth opening, nasolabial flap, Oral submucous fibrosis

INTRODUCTION

Oral submucous fibrosis (OSMF) is a well-recognized condition that has been described since the time of Sushruta as *Vidari*[1]. In modern medical literature, the condition was first described by Schwartz in 1952 as *atrophic idiopathica mucosa oris* [2]. The term "oral submucous fibrosis" was introduced by Pindborg and Sirsat in 1966 and remains in use today.

They defined OSMF as “an insidious, chronic disease that affects any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by or associated with the formation of vesicles, it is always linked to a juxtaepithelial inflammatory reaction followed by fibroelastic change of the lamina propria

and epithelial atrophy, leading to stiffness of the oral mucosa and causing trismus and an inability to eat" [3].

As the disease progresses, it results in difficulty with mastication, phonation, and swallowing. OSMF is most prevalent in the Indian subcontinent, with reported prevalence rates in Southeast Asia ranging from 0.04% to 24.4% [4]. It is classified as a premalignant condition with a notable malignant transformation risk ranging from 1.9% to 7.6% [5].

Research indicates that the etiology of OSMF is multifactorial and complex. Systemic factors such as vitamin B12 deficiency, iron deficiency, zinc deficiency, and anemia contribute alongside local irritants like chilies, chewable tobacco, areca nut, and pan [6]. The earliest clinical symptom is a persistent burning sensation in the buccal mucosa, especially when consuming spicy foods. Clinically, OSMF is divided into three stages: stomatitis, fibrosis, and sequelae. The stomatitis stage is characterized by erythematous areas and vesicles on the buccal mucosa, which rupture and heal with fibrosis. This fibrosis subsequently leads to trismus[7].

Treatment for OSMF has traditionally focused on palliation, primarily aimed at improving mouth opening and alleviating symptoms. Conservative medical treatments are generally ineffective in advanced stages, where surgical intervention becomes necessary. Severe trismus poses a significant surgical challenge. Surgical approaches have evolved, centering on the release of fibrotic bands followed by reconstruction of the resulting defects. Various grafts and flaps have been used for reconstruction, including island palatal mucoperiosteal flaps [8], bilateral tongue flaps [9], split-thickness skin grafts [10], radial forearm free flaps [11,12], buccal fat pad grafts [13], and nasolabial flaps.

The present study aims to compare the efficacy of Buccal Fat Pad (BFP) and Nasolabial Flap (NLF) techniques in the surgical management of oral submucous fibrosis. The primary objectives are to evaluate postoperative mouth opening and oral commissural width following these procedures. The null hypothesis assumes no significant difference exists between the two surgical techniques.

MATERIALS AND METHODS

Study Design, Setting, and Duration: This research was conducted as a randomized, prospective, interventional study featuring a parallel group design with a balanced allocation ratio of 1:1.

Sample Size:

Using G*Power software, with an effect size of 0.80, alpha level set at 0.05, and statistical power of 80%, a total sample size of 20 patients was calculated, with 10 participants allocated to each group.

Eligibility Criteria:

Patients included were those clinically diagnosed with oral submucous fibrosis exhibiting an interincisal mouth opening of less than 20 mm and motivated to discontinue harmful habits. Exclusion criteria encompassed patients with systemic illnesses contraindicating general anesthesia, medically compromised individuals, those unwilling to provide informed consent, and patients presenting with other clinical or radiological causes of trismus.

Procedure:

Patients presenting with complaints of restricted mouth opening, burning sensations, intolerance to spicy foods, or a combination thereof were screened clinically and histopathologically for OSMF. Data collection was standardized using a pre-designed proforma. Informed consent was obtained from all participants. Photographic documentation was performed at baseline. Participants were randomized into two groups via a lottery system: Group 1 underwent surgical management using the nasolabial island flap, while Group 2 was treated with the buccal fat pad graft. Routine hematological investigations and histopathological assessments were conducted preoperatively. All surgeries were performed under general anesthesia via fiberoptic nasotracheal intubation by a single experienced surgeon.

Surgical Technique:

After infiltration of local anesthesia with 1:200,000 epinephrine along the planned incision lines, bilateral intraoral incisions were made at the buccal mucosa level using a number 15 blade and electrocautery, avoiding the Stenson's duct orifice. The incision extended from the oral commissure anteriorly to the anterior pillar of the fauces, soft palate, or pterygomandibular raphe posteriorly, depending on the extent of fibrosis assessed by palpation. Blunt dissection and undermining were performed until fibrous restrictions were completely released.

Nasolabial Flap Reconstruction (Group 1): Following excision of fibrous bands and achievement of adequate mouth opening, third molars were extracted. An elliptical nasolabial flap was designed extending from the nasolabial fold tip to the mandibular border, marked with methylene blue. Flap width ranged between 1.5 and 2 cm, tapering at the ends. The flap was elevated in the plane of the superficial musculoaponeurotic system with preservation of a 1.5-2 cm pedicle near the oral commissure. A transbuccal tunnel was created near the modiolus, and the flap was transposed intraorally without tension. The flap edges were sutured to the defect margins using 3-0 vicryl

sutures. Donor site closure involved subcutaneous undermining and layered closure with 3-0 vicryl and 4-0 ethilon sutures (Figure 1).

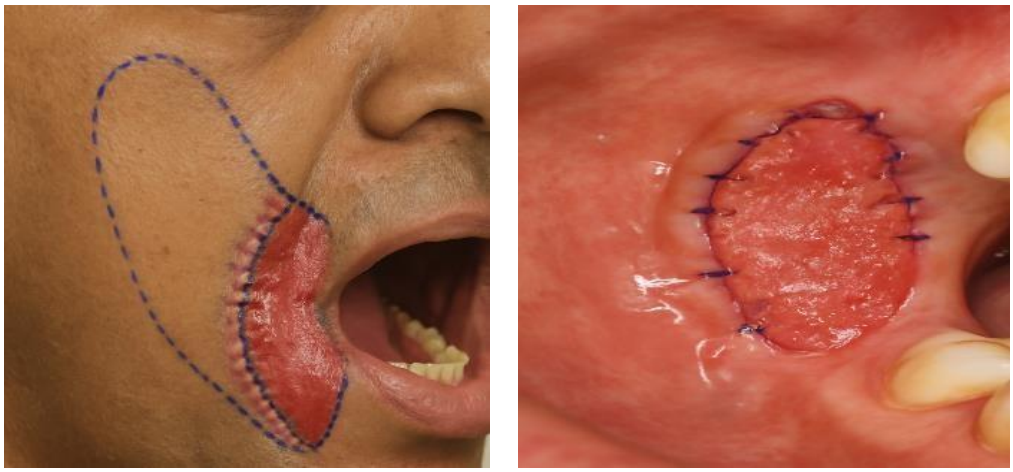


Figure 1. Nasolabial Flap Reconstruction and Clinical Image Showing Nasolabial Flap Coverage of Buccal Mucosal Defect Post-Surgery

Buccal Fat Pad Graft Reconstruction (Group 2)

After fibrous band release and adequate mouth opening, the buccal fat pad was accessed through the posterosuperior margin of the defect. The fat pad was gently teased out via blunt dissection to cover the defect without tension and secured with interrupted and mattress 3-0 vicryl sutures (Figure 2).



Figure 2. Buccal Fat Pad Graft Reconstruction

Postoperative Care and Follow-up

Patients were followed up on postoperative days 7, 15, 30, and 90. Prophylactic antibiotics were administered routinely. Extraoral sutures in Group 1 were removed between days 7 and 10 post-surgery. After the tenth day, patients in both groups commenced rigorous physiotherapy using a Heister's mouth gag, gradually increasing duration and frequency to restore intraoperative mouth opening. Patients were instructed and motivated to continue physiotherapy independently for six months. Interincisal mouth opening was measured using a ruler in millimeters at each follow-up visit.

Statistical Analysis

Data were coded and entered into Microsoft Excel, then analyzed using IBM SPSS Statistics Version 20. Descriptive statistics (means, standard deviations, percentages) were computed. Inter-group comparisons were performed using the unpaired t-test, while intra-group comparisons employed the paired t-test. Statistical significance was set at $p \leq 0.05$.

RESULTS

In groups 1 and 2, mouth opening and commissural width were significantly improved (P <0 .05) compared to the preoperative value as analyzed by paired t-test (Table 1 and Table 2) (Figure 1 and Figure 2).

Inter-group comparison showed that incisal mouth opening and inter-commissural width improved to a greater extent in the nasolabial flap than buccal fat pad and was statistically significant (P <0 .05) (Tables 3 and 4)(Figure 3 and 4).

Table 1. Intra-Group Comparison of Nasolabial Flap

		Mean	Std Deviation	Mean difference	P value
Incisal mouth opening	Pre	10.10	4.012	22.9	0.001(S)
	Post	33.00	3.800		
Commissural width	Pre	42.10	2.906	7.4	0.001(S)
	Post	49.50	4.60072		

S – Statistically significant

Table 2. Intra-Group Comparison of Buccal Fat Pad

		Mean	Std Deviation	Mean difference	P value
Incisal mouth opening	Pre	11.90	1.792	15.7	0.001(S)
	Post	27.60	3.921		
Commissural width	Pre	43.30	3.59166	1.1	0.001(S)
	Post	44.40	3.53396		

S – Statistically significant

Table 3. Inter Group Comparison of Incisal mouth opening

		Mean	Std Deviation	Mean difference	P value
Pre	Group 1	10.10	4.012	1.8	0.21
	Group 2	11.90	1.792		
Post	Group 1	33.00	3.800	5.4	0.006(S)
	Group 2	27.60	3.921		

S – Statistically significant

Table 4. Inter Group Comparison of Commissural Width

		Mean	Std Deviation	Mean difference	P value
Pre	Group 1	42.100	3.63	1.2	0.46
	Group 2	43.300	3.59		
Post	Group 1	49.500	4.60	5.1	0.01(S)
	Group 2	44.400	3.23		

S – Statistically significant

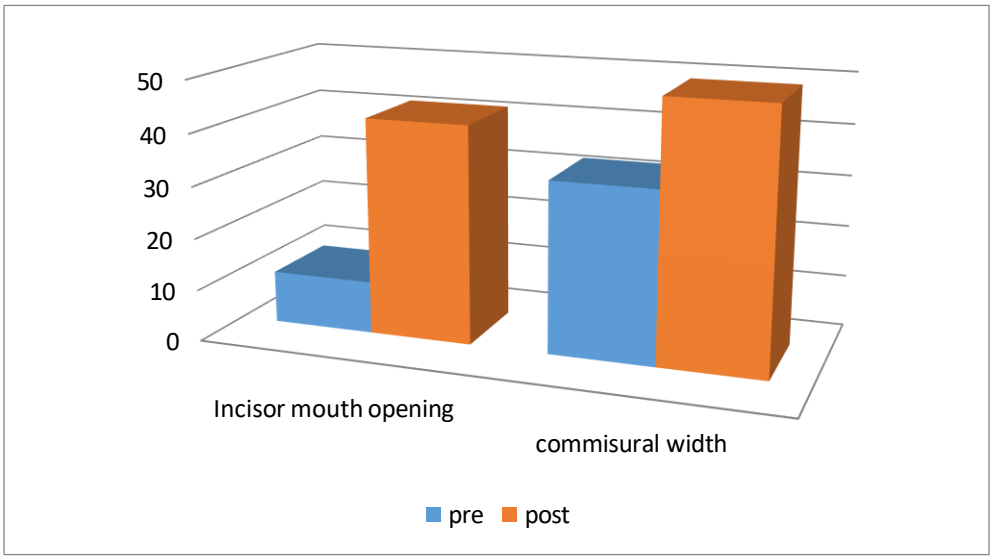


Figure 1. Intra-Group Comparison of Nasolabial Flap

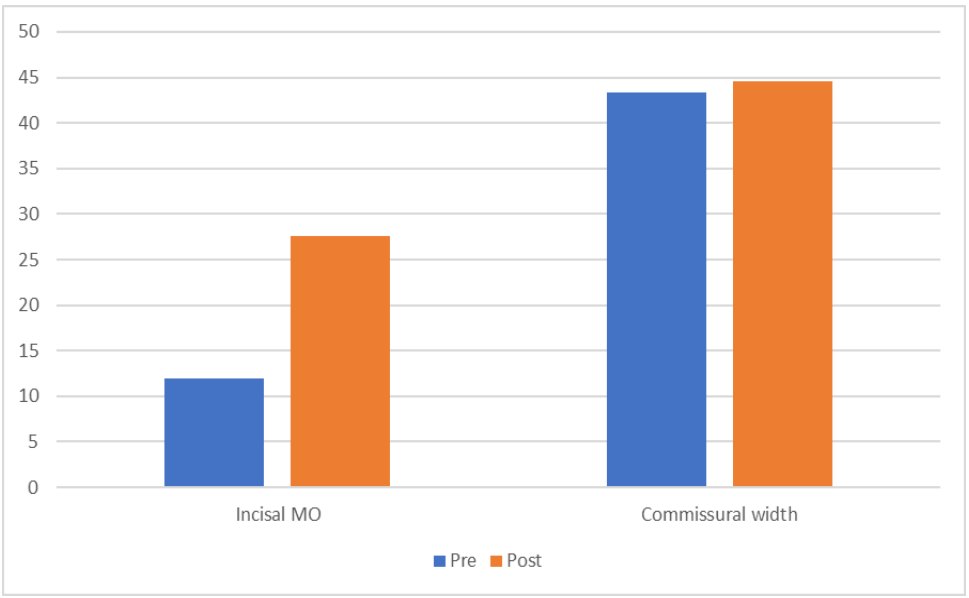


Figure 2. Intra Group Comparison of Buccal Fat Pad

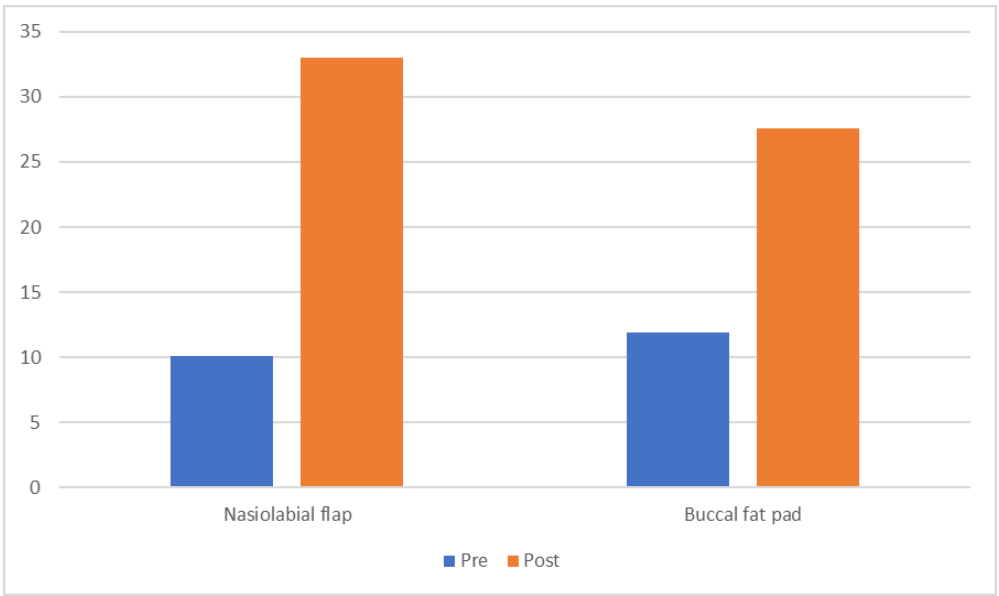


Figure 3. Inter group comparison of incisal mouth opening

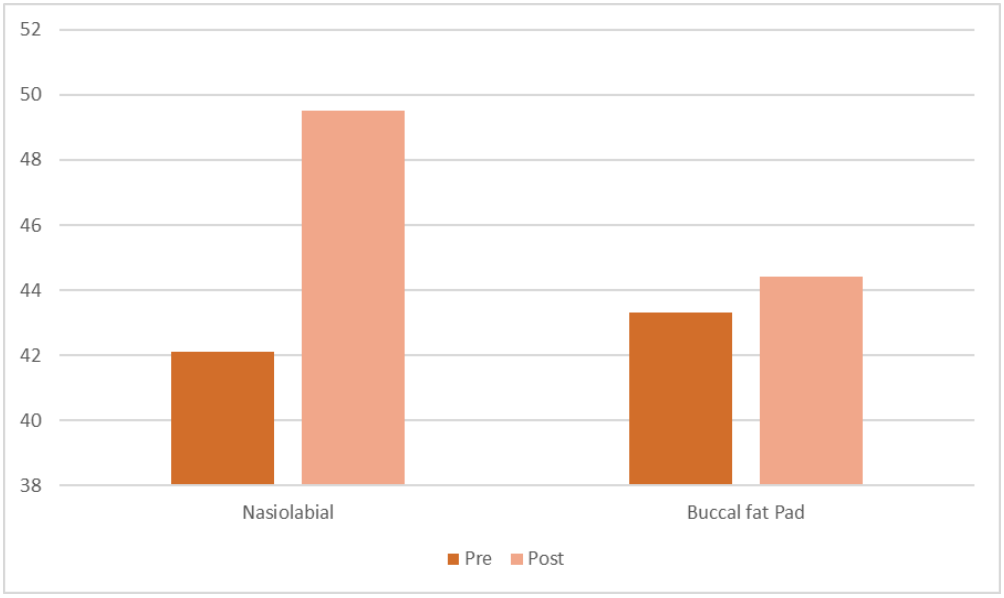


Figure 4. Inter-Group Comparison of Commissural Width

DISCUSSION

Oral submucous fibrosis (OSMF) is a chronic, progressive, scarring disorder recognized as a precancerous condition, primarily linked to the habitual chewing of betel quid[15]. The management of OSMF encompasses various therapeutic strategies including habit cessation, pharmacological treatments, surgical intervention, and oral physiotherapy. Conservative treatments such as intralesional injections of hyaluronidase, placental extracts, chymotrypsin, interferon-gamma, curcumin, pentoxifylline, and antioxidants like lycopene are typically effective for patients with early-stage (grades 1 and 2) OSMF. However, patients with advanced disease (grades 3 and 4), characterized by mouth opening less than 25 mm, often require surgical correction [16].

Surgical management involves excision of fibrotic bands under general anesthesia, followed by defect reconstruction using various flap techniques. The nasolabial flap (NLF) and buccal fat pad (BFP) flap are among the most commonly employed reconstructive options. The nasolabial flap is favored for anterior oral and maxillofacial defect reconstruction due to its robust vascular supply via extensive anastomoses. Despite its advantages, the NLF carries potential complications including intraoral hair growth, visible extraoral scarring, orocutaneous fistula formation, hematoma, infection, and hypertrophic scarring, especially when donor site closure is performed under tension [16].

Pravin Lambade et al. 2016 [17] conducted a prospective two-year study involving 20 OSMF patients with mouth openings less than 16 mm who underwent fibrotomy defect reconstruction using NLF. Intraoperative mouth openings ranged from 32 to 44 mm, and postoperative follow-up revealed mouth openings between 20 and 44 mm after two years. Early postoperative complications included prominent intraoral hair growth and visible extraoral scars. Similarly, Qayyum MU et al. 2018[18] reported on the use of nasolabial and extended nasolabial flaps for oral defect reconstruction in OSMF, noting preoperative mouth openings from 5 to 16 mm that increased to 29 to 39 mm at six months postoperatively. Some complications such as poor scarring and flap trauma from wisdom teeth were observed but managed effectively.

The buccal fat pad graft has gained popularity for reconstructing small to medium intraoral defects involving the palate, gingival sulcus, alveolus, and buccal mucosa. It is a straightforward, reliable, and convenient graft that requires no secondary surgical site, offers excellent vascularity, and is resistant to infection, with minimal associated disadvantages [16]. Saravanan et al.2012[19] reported on eight OSMF cases treated with BFP grafts. Patients presented with preoperative mouth openings ranging from 3 to 18 mm, underwent coronoidectomy, and achieved intraoperative openings of 25 to 38 mm. Postoperative mouth openings ranged from 25 to 36 mm. The grafts healed uneventfully and epithelialized within 3–4 weeks. The authors concluded that the BFP is a suitable interpositional graft due to its anatomic position, rich blood supply, and ease of harvest and mobilization.

Our current comparative analysis rejects the null

hypothesis, indicating a significant difference between NLF and BFP in surgical defect closure for OSMF. At three months postoperatively, Group 1 (NLF) showed an average mouth opening increase of 1.8 mm, whereas Group 2 (BFP) showed an increase of 5.4 mm ($p < 0.05$). Similarly, postoperative commissural width increased by 1.2 mm in Group 1 and 5.1 mm in Group 2, both statistically significant ($p < 0.05$). Thus, for patients with OSMF, BFP demonstrated superior functional outcomes compared to NLF.

Several previous studies support our findings. Lathi et al.2022 [20] and Anehosur et al. 2020[21] observed comparable results; Lathi et al. reported a mean postoperative incisal opening increase of 22.9 mm and intercommissural width increase of 7.4 mm following NLF, whereas the BFP group had a mean mouth opening increase of 15.7 mm with negligible commissural width change. Their conclusion favored NLF as the superior interpositional material in stage IV OSMF cases for minimizing relapse, reserving BFP for stage III cases. Anehosur et al. found significant postoperative increases in interincisal mouth opening and commissural width favoring the NLF group compared to the BFP group.

Conversely, studies by Rai et al. 2013[22] and Sikkerimath et al. 2020[23] reported differing outcomes, with BFP yielding better results. Rai et al. documented mean postoperative mouth openings of 32 mm for NLF and 29 mm for BFP groups, with no significant differences in commissural widths. They noted higher complication rates with NLF, including partial flap necrosis, oral commissure widening, and temporomandibular joint subluxation. BFP avoided issues such as extraoral scarring and intraoral hair growth. Sikkerimath et al. reported postoperative mouth openings of 40 mm and 34.7 mm for BFP and NLF groups, respectively, concluding that BFP provided superior outcomes regarding mouth opening and complications.

A systematic review by Basu et al. [16] analyzed five studies involving 484 patients (BFP=224, NLF=260), reporting mean postoperative mouth openings of 29.8 mm for BFP and 31.9 mm for NLF over an average follow-up of 26.4 months. Another meta-analysis by Wadde KA et al.2024[24] reviewed data from 152 patients, finding that BFP achieved 1.41 times greater maximal interincisal opening and 0.25 times more improvement in commissural width than NLF, favoring BFP in OSMF treatment.

Agrawal et al.2018[25] and Pardeshi et al. [26] recommended BFP for mild trismus cases (mouth opening >15 mm) and NLF for severe trismus (<15 mm) due to the latter's bulk. Ambereen et al.2019[27] suggested combining both techniques, using BFP for posterior oral cavity reconstruction and NLF for anterior defects in OSMF.

CONCLUSION

The findings from this study, when compared with the existing literature, suggest that the nasolabial flap offers better results as an interpositional graft in enhancing both incisal mouth opening and commissural width. Nonetheless, more extensive research with larger sample sizes and longer follow-up durations is needed to more thoroughly evaluate surgical treatments for oral submucous fibrosis. Moreover, future investigations should also focus on additional factors such as wound healing, facial appearance, and postoperative complications to provide a more comprehensive assessment of these grafting methods.

DECLARATIONS

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Ethical Approval
"Not applicable"

Consent for publication
"Not applicable"

Ethical approval
None

Competing interest
The authors declare that there are no competing interest.

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CLINICAL CASE SERIES

USES OF LATERAL BONE WINDOW REPOSITIONING TECHNIQUE IN MAXILLARY LATERAL SINUS ELEVATION: A CLINICAL AND RADIOGRAPHIC FOLLOW-UP STUDY FOR 121 PATIENTS FOR 30 TO 90 MONTHS.

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ABSTRACT

Background: Maxillary lateral sinus elevation (LSE) is crucial for increasing vertical bone height in the posterior atrophic maxilla. It facilitates implant placement by creating adequate bone support. This case series describes the lateral bone window repositioning (WR) technique in LSE and evaluates its effectiveness in graft stabilization and clinical success through clinical and radiographic examination.

Methods: This case series included 121 patients (151 sinuses) who met specific inclusion criteria. A total of 285 implants were placed: 150 (52.6%) implants were inserted simultaneously, while 135 (47.4%) were placed 4–6 months after the MLSE procedure. All cases were followed clinically and radiographically for 30 to 90 months. Preoperative cone beam computed tomography (CBCT) scans were taken for proper evaluation of the surgical site, and postoperative CBCT scans were performed 4–6 months after LSE. Patients underwent follow-up evaluations every six months for clinical implant assessment, and periapical images were taken annually to monitor marginal bone levels. The surgical protocol involved performing a beveled osteotomy using a piezoelectric device to create a bone lid. After removal, the Schneiderian membrane was elevated to manage space for the bone graft. The bone lid was then repositioned in its original place.

Results: Among 151 LSE cases, 146 (96.7%) exhibited successful outcomes, with complete integration of the bone lid into the surrounding bone, confirmed through clinical and radiological examinations. Three failures were reported, one due to trauma and two resulting from sinus infections. Two cases were excluded due to bone window fractures during removal. Small perforations of the Schneiderian membrane occurred in 28 cases (18.5%), all successfully managed with collagen membranes.

Conclusion: The WR technique in LSE demonstrated a high success rate, repeatable results, and effective graft integration, making it a viable option for vertical bone height augmentation.

Keywords: Maxillary sinus elevation, bone window repositioning, graft stabilization, dental implants, Schneiderian membrane, bone augmentation

1. INTRODUCTION

Loss of posterior maxillary bone height is often due to physiological remodeling following tooth loss, sinus pneumatization, or both[1,2]. Various bone augmentation techniques have been developed to facilitate implant placement, including maxillary lateral sinus elevation, crestal access, and vertical osteotome-mediated sinus floor elevation. LSE is recommended for cases with ≤5 mm of subantral vertical bone height, allowing simultaneous or delayed implant placement [3–5]. Initially proposed by Tatum [6] and later

detailed by Boyne and James in 1980(7), LSE has evolved into a safe treatment modality with a low complication rate[8]. The procedure involves creating a lateral window in the sinus wall to elevate the Schneiderian membrane, filling the created space with a bone graft or blood clot [9]. The lateral bone window can either remain attached to the membrane and rotated inward or be entirely removed and replaced at its original site [10].

Covering the graft material with a membrane has been shown to enhance healing by preventing graft migration and reducing soft tissue invasion[11]. Lundgren et.al. described the WR technique in LSE. That technique consists of repositioning the removed bone plate to its original site after LSE [12]. As an autogenous barrier, the bone window will enhance new bone formation and graft integration [13]. This case series aims to evaluate the validity of WR in LSE through radiographic analysis and clinical assessment.

MATERIALS AND METHODS

After the initial clinical evaluation, a CBCT examination was performed for preoperative assessment. Four to six months after the surgery, a second CBCT examination was taken to evaluate bone height and to schedule implant placement if not done simultaneously.

Inclusion and Exclusion Criteria

Inclusion Criteria:

Participants were included in the study based on the following conditions:

1. Partial or complete edentulism in the maxillary molar region, with a residual ridge height of ≤ 5 mm.
2. Age over 20 years.
3. Overall good general health.
4. Satisfactory oral hygiene and periodontal status suitable for implant placement.
5. Willingness to provide informed consent.

Exclusion Criteria:

The following factors led to exclusion from the study:

1. Systemic conditions that contraindicate dental surgery, including uncontrolled metabolic disorders, history of head or neck radiotherapy, chemotherapy, or immunosuppressive therapy.
2. Local nasal pathologies that preclude maxillary lateral sinus elevation (MLSE).
3. Presence of untreated periodontal disease.
4. Heavy smoking (defined as more than 10 cigarettes per day); 5 patients were excluded on this basis.

A total of 121 patients, accounting for 151 sinuses, fulfilled these criteria and were included in the study.

Surgical Procedure

All surgeries were performed by the same trained oral surgeon (H.A.H.) and his surgical team. Local anesthesia (lidocaine with adrenaline 1:100,000) was administered, and a mucoperiosteal flap was elevated to expose the lateral sinus wall. A beveled osteotomy was performed using a Piezosurgery® touch unit (Mectron S.p.A., Genova, Italy) with specific bone tips (OT7A, OT8L, OT8R) to create a trapezoidal bone window measuring approximately 15–20 mm mesiodistally and 6–10 mm apico-coronally.

The bone window was carefully removed to avoid damage to the Schneiderian membrane, which was then elevated using sinus curettes. When residual bone height was ≤2 mm, implant placement was delayed. In 150 cases (54.3%), implants were placed simultaneously. A 50:50 mixture of autogenous bone (harvested from the tuberosity and zygomatic area using a bone scraper) and xenograft bone substitute (Bio-Oss® L) was grafted while minimizing pressure on the sinus membrane. The preserved bone lid was repositioned, and the flap was sutured using 4.0 absorbable sutures.

Postoperative care included a prescription of antibiotics (amoxicillin 500 mg TID for 7 days), NSAIDs (ibuprofen 400 mg BID for 7 days), and 0.12% chlorhexidine mouth rinse TID for 14 days. Sutures were removed after 14 days. In cases of membrane perforation, medication duration was extended to 14 days, and patients were instructed to avoid blowing their noses, coughing, or sneezing forcefully for 15 days.



Figure1. Surgical procedure A) Flap access and the bone lid removed B) Schneiderian membrane elevation and placement of bone graft with simultaneous implant placement C) The bone lid repositioned.

Radiographic Examination

Preoperative CBCT scans were taken to assess residual bone. A second CBCT scan was performed 4–6 months postoperatively to evaluate new bone formation. Bone height was measured on coronal and sagittal reconstructions while bone width was evaluated using axial reconstructions. Linear measurements were taken from the alveolar ridge base to the highest point of the graft. Bone window integration was assessed and defined as a complete bony union between the bone lid and surrounding bone with no evidence of radiolucency.

All preoperative CBCT assessments were conducted using the **PaX-i3D SMART** CBCT system, with a **Field of View (FOV) of 8 × 8 cm or 10 × 10 cm**, optimized for bone height and ostium patency analysis. Standard resolution imaging was achieved with a voxel size of 0.2–0.3 mm, while exposure settings were maintained at 80–100 kept, 5–10 mA, with a scan time of 10–20 seconds.

Postoperative CBCT scans were performed 4–6 months after surgery to assess graft volume and stability, new bone formation, Schneiderian membrane integrity, and ostium patency. The same CBCT machine and imaging parameters were used for consistency across all patients.

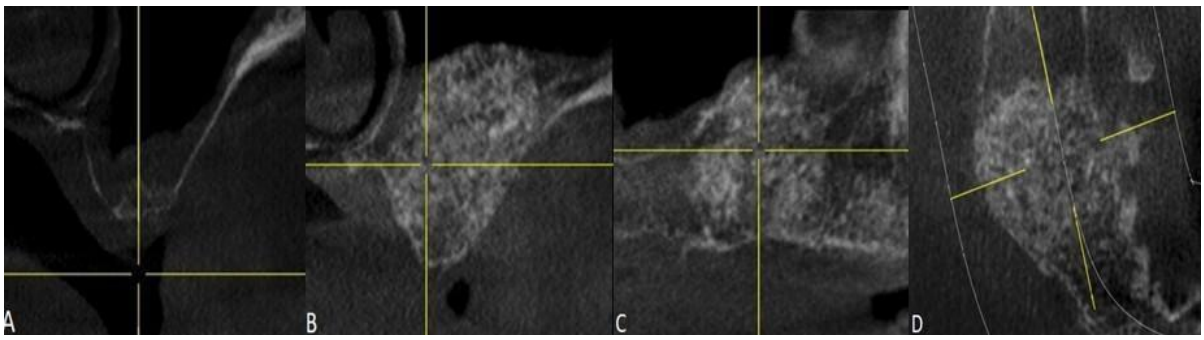


Figure 2. A) Preoperative frontal view of the surgical site. B) Bone height evaluation on the coronal plane. C) Graft height evaluation on the sagittal plane. D) Graft width on axial plane.

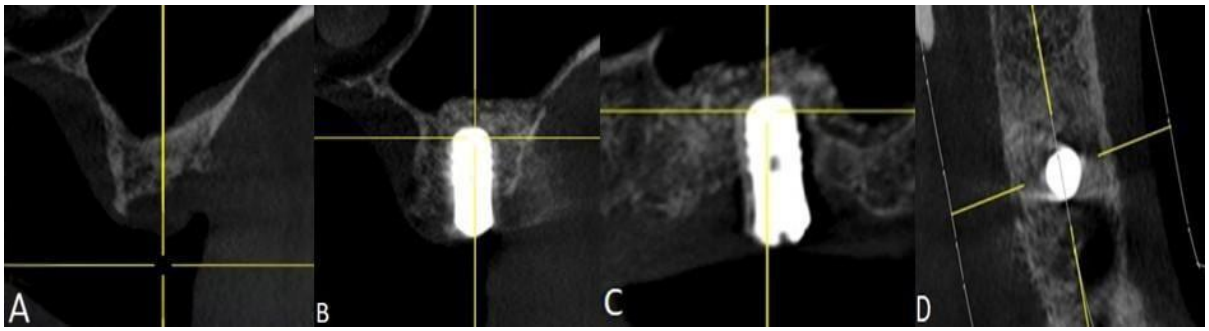


Figure 3. A) Preoperative frontal view of the surgical site. B) Frontal view of the surgical site showing bone graft with simultaneous implant placement. C) Sagittal view of the surgical site showing bone graft with simultaneous implant placement. D) Axial view of the surgical site showing bone graft with simultaneous implant placement.

Clinical Examination

During implant placement or uncovering surgery, the surgical site was clinically examined for proper integration of the bone lid. Signs of infection, swelling, soft tissue dehiscence, and mobility of the bone lid were noted as potential

complications. Patients were followed every six months post-implant loading to monitor implant stability, pocket formation, mobility, and signs of infection or discomfort.

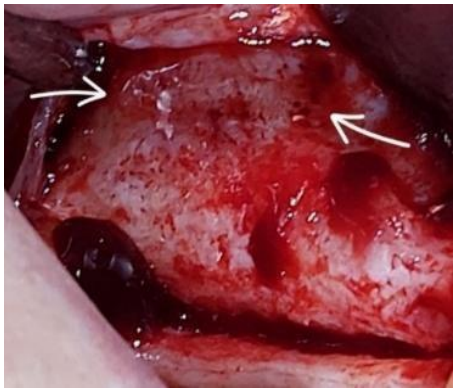


Figure 4. Complete integration of the bone lid with the surrounding bone.

RESULTS

A total of 121 patients (75 women (62%) and 46 men (38%)), aged 36–68 years (mean age: 54.2 ± 12.3 years) underwent 151 LSE procedures between June 2014 and December 2021. The follow-up durations ranged from 30 to 90 months, with the majority of patients (91 out of 121, or 75,2%) having a follow-up of 60 months. Thirty patients required bilateral sinus elevation. Five patients were excluded because of heavy smoking.

A total of 285 implants were placed: 200 Cowell Medi (KOREA Inc.) implants (66.7%) and 85 Bone Level Tapered and Tissue Level Straumann® (Basel, Switzerland) implants (33.3%). Among them, 150 (52.6%) implants were inserted simultaneously, while 135 (47.4%) were placed 4–6 months after the LSE procedure. Bone lid integration was confirmed via reentry and radiographic evaluation. The overall success rate of the WR technique was 96.7%. Of 151 cases, 146 exhibited successful bone lid integration without fibrous connective tissue invasion. Three failures were reported (one due to trauma, two (1.32%) due to sinus infections), and two cases were excluded due to bone window fractures. Schneiderian membrane small perforations were observed in 28 cases (18.54%), 17 of which were detected at the time of window separation. Of the 28 membrane perforations, all were managed using a Jason® native pericardium collagen membrane (Botiss Biomaterials GmbH, Zossen, Germany). No surgeries were aborted, no implant failures were recorded, nor any difference in implant success rates or bone integration compared to cases without perforations. No significant intraoperative or postoperative complications, such as severe hematoma or excessive swelling, were observed. The post-operative CBCT scans, taken four to six months after surgery, revealed bone resorption ranging from 8 to 12%.

TABLE 1. Sample characteristics and results

Variable	N	Mean ± SD / Percentage
Age (years)	121	54.2 ± 12.3
Female Gender	121	62%
Simultaneous Implants	151	52.60%
Delayed Implants	151	47.40%
Membrane Perforations	151	18.54%
Bone Window Fractures	151	1.32%
Implant Success Rate	151	96.70%
Mean Follow-Up (months)	121	59.17
Median Follow-Up (months)	121	60

DISCUSSION

Cone Beam Computed Tomography (CBCT) has become an indispensable tool in modern implantology, particularly for evaluating the maxillary sinus before and after sinus lift procedures. Its high-resolution, three-dimensional imaging provides critical insights into bone volume, density, and anatomical variations, aiding clinicians in surgical planning.

Before a sinus lift, a comprehensive CBCT scan is essential to evaluate several factors influencing the procedure's success:

- **Bone Height and Width**: CBCT enables precise measurement of the residual alveolar bone between the crest and sinus floor.

- **Maxillary Sinus Anatomy**: It helps identify anatomical variations, such as septa, which may complicate the procedure.
- **Schneiderian Membrane Thickness**: Assessing membrane integrity and thickness is crucial to prevent perforation.
- **Ostium and Sinus Health**: Evaluating sinus drainage pathways is essential, as blocked or inflamed sinuses increase the risk of postoperative complications.
- **Proximity to Vital Structures**: CBCT assists in mapping the infraorbital nerve and adjacent anatomical landmarks to minimize surgical risks.

A **cone beam computed tomography (CBCT) scan** after **surgery,** is used to evaluate the procedure and check for potential complications.

- Confirms that the bone graft material is properly placed.
- Assess the height and density of the newly augmented sinus floor.
- Determines if the graft has maintained its volume or if any resorption has occurred.
- Detects sinus membrane perforations.
- Identifies fluid accumulation or sinus infections (sinusitis).
- Checks for bone particles displacing into the sinus cavity.
- Measures the available bone height and width.
- Assesses bone density to determine implant stability.
- Helps in precise implant placement by avoiding anatomical structures like the maxillary sinus and nerves.
- Monitors the bone healing process over time.
- Determines the readiness for implant placement if the lift was done as a staged procedure.

In our case series, CBCT exams were done before the LSE and after 4 to 6 months. Our primary goals were the assessment of bone height, sinus anatomy, and health before and after the sinus lift procedures.

Animal models showed an acceleration of bone formation with better quality and ossification of the gap between the lid and the surrounding bone[14]. In clinical studies, the bone lid without grafting material showed adequate bone formation[15]. Lundgren et al. hypothesized that the healing process when using the bone lid technique involves the prevention of mucosal growth towards the sinus, the preservation of the maxillary sinus pneumatic conditions, and finally the blood clot stabilization and promotion of the bone formation[12]. Repositioning the removed bone window eliminates the need for additional membrane placement while providing an autogenous barrier that enhances graft stability, soft tissue migration, and new bone formation which contributes to high implant success rates[9-16]. Compared to other methods, such as window rotation or complete removal, the repositioned bone lid maintains a natural osteogenic environment and shows better osteoinductivity properties, facilitating predictable healing[18]. Using the lateral wall bone for the two-dimensional reconstruction of the severely resorbed maxilla by creating a box shape, and providing an architectural design for the augmented area confirms the osteoconductivity properties of the bone lid[19]. Jia et al. compared the lateral sinus lift with and without the bone lid repositioning with immediate implant placement, and found a 100% implant survival rate, increased alveolar ridge height, and apical bone gain in the group treated with the bone lid repositioning compared with the use of growth factors concentrated membrane[20].

Additionally, the use of piezoelectric surgery ensures precise beveled bone cuts, reducing the risk of Schneiderian membrane perforation and improving overall surgical outcomes[21]. It also provides a self-retentive characteristic for the bone lid, thus eliminating the need for rigid fixation[13,22,25]. Created margins should be as thin as possible for a better repositioning of the bone lid[26]. Non-rigid fixation methods, such as fibrin or cyanoacrylate-based surgical glue, can also be used[16]. While minor complications, such as membrane perforations were encountered in this study, all cases were successfully managed without compromising results. In all the follow-up periods, no difference was found between cases with or without membrane perforation.

Despite this case series providing strong evidence for the viability of the WR technique, this study is limited by its case series design, which lacks a control group. Therefore, while we can report on the outcomes of the WR technique, we cannot directly compare its effectiveness to other LSE techniques like complete window removal or window rotation with or without using a membrane to close the sinus window.

CONCLUSION

Within the study's limitations in this case series, the WR technique in LSE showed a high success rate and predictable results in terms of vertical bone height increase with minor complications. It offers a cost-effective and viable alternative to other procedures. Additional randomized controlled trials could provide stronger confirmation of our results.

DECLARATIONS

Competing interests

The authors have declared that no competing interests exist.

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ORIGINAL ARTICLE

PROGNOSTIC INDICATORS OF VERRUCOUS CARCINOMA - A SEVEN YEAR INSTITUTIONAL STUDY

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Verrucous carcinoma (VC), a rare subtype of squamous cell carcinoma (SCC), is distinct due to its unique clinical and histopathological characteristics Unlike conventional SCC, VC is characterized by a slow growth rate, locally aggressive behaviour, and a limited tendency for distant metastasis. However, it remains a clinical concern due to its potential to transform into invasive SCC. Materials and Methods:The research focused on cases diagnosed between 2017 and 2024, involving patients with verrucous carcinoma (VC), verrucous carcinoma transforming into squamous cell carcinoma (SCC), and proliferative verrucous leukoplakia. The hospital's database served as the primary source of patient data for this study, offering access to clinical records, histopathological findings, and other relevant diagnostic information.Results : Out of 60 cases in the dataset, 58 (96.7%) were valid and included in the analysis, while 2 cases (3.3%) had missing data. This high percentage of valid cases ensures the reliability of the findings.

Keywords*:* Verrucous carcinoma, oral cancer, prognosis

INTRODUCTION

Head and neck cancers constitute a significant global health challenge, ranking as the seventh most prevalent cancer worldwide. Approximately 90% of these malignancies arise within the oral cavity, predominantly affecting the squamous epithelium. Verrucous carcinoma (VC), a rare subtype of squamous cell carcinoma (SCC), is distinct due to its unique clinical and histopathological characteristics[1]. Unlike conventional SCC, VC is characterized by a slow growth rate, locally aggressive behaviour, and a limited tendency for distant metastasis. However, it remains a clinical concern due to its potential to transform into invasive SCC. Understanding the prognostic factors associated with VC is critical for guiding treatment approaches and improving patient outcomes [2].

The incidence of verrucous carcinoma is relatively low, accounting for less than 5% of all oral cancers. It is most commonly diagnosed in individuals over the age of 40, with a slight male predominance. Its geographic distribution is influenced by cultural and lifestyle factors, such as the use of smokeless tobacco and betel quid chewing. In regions like Southeast Asia and the Indian subcontinent, these habits significantly contribute to the prevalence of VC. Conversely, in Western countries, the use of smokeless tobacco products plays a prominent role in its etiology. Though typically observed in older adults, occasional cases in younger individuals highlight the need for awareness across all age groups [3].

VC is primarily associated with chronic mucosal irritation. Tobacco use, both in smoked and smokeless forms, remains the most significant risk factor. Other contributory factors include areca nut chewing, alcohol consumption, and poor oral hygiene[3].Human papillomavirus (HPV) infection has emerged as a potential etiological agent, with studies reporting varying prevalence rates depending on the diagnostic techniques used, such as polymerase chain reaction (PCR) or immunohistochemistry. The interaction between HPV and host cellular mechanisms is believed to play a role in the development and progression of VC. Additionally, genetic predispositions and chronic inflammatory conditions may further exacerbate its pathogenesis[4].

Clinically, VC often presents as a slow-growing, exophytic lesion with a warty or cauliflower-like surface.

Verrucous carcinoma lesions are almost always large, exophytic, soft, fungating, slow growing neoplasms with a pebbly mamillated surface. These lesions commonly involve the buccal mucosa, gingiva, tongue, and other areas of the oral cavity. Early-stage VC may mimic benign conditions such as verrucous leukoplakia, making accurate diagnosis challenging. Patients are typically asymptomatic in the initial stages, delaying diagnosis until the lesion becomes extensive. Differential diagnoses include papillomas, leukoplakia, and conventional SCC, necessitating a thorough clinical and histopathological evaluation [5,6].

Histologically, VC is characterized by hyperkeratosis, papillomatosis, and a pushing border of invasion without infiltrative growth. Cellular atypia is minimal, and mitotic activity is generally absent, reflecting the tumours well-differentiated nature[7]. Immunohistochemical markers such as p53, Ki-67, and cytokeratins can assist in distinguishing VC from other oral lesions. The unique histopathological features underscore the importance of expert pathology review for accurate diagnosis and effective management [8,9].

The prognosis for VC is generally favourable when detected early and managed appropriately. The absence of metastatic potential to regional lymph nodes and distant organs distinguishes VC from other forms of SCC. Prognostic factors influencing outcomes include tumor size, depth of invasion, anatomical location, and HPV status. Overexpression of molecular markers such as p53 and high Ki-67 proliferation indices has been linked to aggressive disease behaviour. Additionally, host- related factors such as age, immune competence, and the presence of comorbidities play crucial roles in determining prognosis [10].

Recent advancements in diagnostic techniques have improved the detection and management of VC. High-resolution imaging modalities, including magnetic resonance imaging (MRI) and positron emission tomography-computed tomography (PET-CT), provide detailed insights into tumor extent and involvement of adjacent structures[11]. Molecular profiling has enabled the identification of genetic and epigenetic alterations, offering potential biomarkers for targeted therapy. These innovations have enhanced diagnostic accuracy and facilitated personalized treatment planning [12].

Surgical excision with clear margins remains the cornerstone of VC management. In cases where complete excision is challenging, adjunctive therapies such as radiotherapy and chemotherapy may be employed. However, the role of these therapies is limited due to the tumours low radio sensitivity and chemo resistance[13]. Emerging treatments, including targeted therapy and immunotherapy, are being explored in clinical trials, showing promise in improving outcomes for specific patient subsets. The integration of these novel approaches into clinical practice could revolutionize the management of VC in the future [14].

This study aims to investigate the prognostic indicators of verrucous carcinoma through a comprehensive analysis of cases diagnosed at Saveetha Dental College and Hospitals between 2017 and 2024.

MATERIALS AND METHODS

This study was a retrospective analysis conducted at the Department of Oral Pathology, Saveetha Dental College and Hospitals. The research focused on cases diagnosed between 2017 and 2024, involving patients with verrucous carcinoma (VC), verrucous carcinoma transforming into squamous cell carcinoma (SCC), and proliferative verrucous leukoplakia. The hospital's database served as the primary source of patient data for this study, offering access to clinical records, histopathological findings, and other relevant diagnostic information.

The study included patients diagnosed histopathologically with oral VC, cases showing a transformation from VC to invasive SCC, and proliferative verrucous leukoplakia. Patients with incomplete medical records, those whose diagnosis was not confirmed by histopathological analysis, and those undergoing treatment outside the hospital were excluded to ensure consistency and reliability in data collection.

Clinical data, including patient demographics (age, gender, and lifestyle habits such as tobacco or areca nut use) , lesion characteristics (site, size, and clinical presentation), and duration of symptoms, were extracted. Information regarding treatment modalities, recurrence rates, and follow-up outcomes was also gathered. Provided in Table 1.

The data were compiled and tabulated using Microsoft Excel.

All cases were reviewed by a team of experienced pathologists to confirm the diagnosis of VC and related conditions. Histopathological analysis focused on key features, including hyperkeratosis, exophytic growth, papillomatosis, and the characteristic "pushing" border of invasion. Data were analyzed using SPSS software. Descriptive statistics were calculated to summarize patient demographics and clinical features. Categorical variables were expressed as frequencies and percentages, while continuous variables were presented as means and standard deviations.

Comparative analyses were conducted to evaluate the correlation between clinical features and prognostic outcomes. Statistical significance was determined using appropriate tests, such as the chi-square test for categorical variables and t-tests for continuous variables, with a p-value of <0.05 considered statistically significant.

Table 1. Clinical features

Variable	Category	No. of Patients
Gender	Male	39
	Female	7
Age	≤50 years	16
	≥51 years	37
Habits	With Habits	35
	Without Habits	26
Laterality	Left	29
	Right	19
	Bilateral	0
Lymph Node Metastasis	Yes	41
	No	20
TNM Stage	T1	2
	T2	2
	T3	1
	T4	11
Invasion	Maxilla	5
	Mandible	3
	Skin	1

RESULTS

The statistical analysis of verrucous carcinoma and related conditions provides significant insights into the relationships between demographic factors and clinical outcomes. Out of 60 cases in the dataset, 58 (96.7%) were valid and included in the analysis, while 2 cases (3.3%) had missing data. This high percentage of valid cases ensures the reliability of the findings. The analysis revealed a strong association between age and advanced disease features such as metastasis and extranodal extension (ENE), with older patients showing a higher likelihood of presenting with these characteristics ($p < 0.001$). This suggests that disease progression and spread are more prevalent in older individuals, underscoring the need for vigilant monitoring and aggressive management in this group. Additionally, a significant relationship was identified between gender and habits like smoking or chewing tobacco ($p = 0.045$), indicating that these behaviours, known risk factors for carcinogenesis, are more prevalent in one gender. However, no significant associations were found between age and diagnosis type, suggesting that the type of verrucous carcinoma or related condition is distributed uniformly across different age groups. Similarly, no significant correlations were observed between age and habits, or gender and clinical outcomes such as metastasis or ENE. The analysis also found no gender-specific differences in biopsy results or diagnosis types, indicating that the patterns of disease presentation and diagnostic findings are similar across genders.

These findings have important clinical and public health implications. The strong association between age and advanced disease characteristics highlights the importance of early detection and tailored treatment plans for older patients, who are at greater risk of

metastasis and ENE. Targeted interventions, such as more frequent screenings and comprehensive management approaches, could help mitigate disease progression in this high-risk group. The significant link between gender and habits underscores the need for gender-specific public health strategies, such as tobacco cessation programs, to address modifiable risk factors and reduce disease burden.

In contrast, the absence of significant associations between other variables, such as age and diagnosis type or gender and metastasis, suggests that certain factors may not play a pivotal role in disease progression or outcomes.

DISCUSSION

Verrucous carcinoma (VC) is a rare variant of squamous cell carcinoma (SCC) that predominantly affects the oral cavity, particularly the buccal mucosa, gingiva, and tongue. Our study, comprising 61 cases, revealed a male predominance (44 males, 13 females), consistent with previous literature indicating that VC is more common in males, particularly in regions with high tobacco consumption. Another study conducted between 2018 and 2022, involving 5,469 biopsy-proven head and neck cancer patients, found a male-to-female ratio of 4.2:1. The study highlighted that men constituted 80.80% of the study population, with oral cavity lesions being the most common site. The higher prevalence in males was linked to greater exposure to risk factors such as tobacco and alcohol[15]. Similarly, a study by Batsakis et al. (2019) reported that male patients accounted for approximately 75% of VC cases, aligning with our findings[16].

Similarly, a study focusing on oral verrucous carcinoma over a ten-year period reported that 83% of the patients were male, resulting in a male-to-female ratio of 5:1. The mean age at presentation was 49 years. In this cohort, 33.3% of patients chewed tobacco, 26.6% both chewed and smoked tobacco, and 10% only smoked tobacco. This further underscores the significant role of tobacco use in the etiology of VC, especially among males17.

Our data showed 35 cases (57.3%) with a history of deleterious habits, with pan chewing (11 cases), gutka (3 cases), and smoking (2 cases) being the most frequently reported. The association of VC with tobacco and areca nut has been well documented in prior studies. Ackerman first provided a comprehensive description of verrucous carcinoma in 1948, highlighting its association with tobacco use. In his study of 31 patients, Ackerman noted that many were elderly men with a history of chewing tobacco[18]. This led him to postulate a link between tobacco use and the development of verrucous carcinoma[19].

More studies by Munde et al. (2013) reinforced that 80-90% of patients with VC have a history of tobacco use, either in the form of chewing or smoking[20].

A study by Ackerman found that habit-associated VC cases showed a higher prevalence of field cancerization, where multiple lesions develop due to long-term carcinogen exposure[21]. Our findings support this, as many patients with habitual risk factors presented with lesions in high-risk areas such as the buccal mucosa and gingiva. In contrast, a study by Hashibe et al. (2003) demonstrated that alcohol alone is not a strong independent risk factor, but when combined with tobacco, it significantly increases the risk[22].

This was evident in our cohort, where alcohol use was observed in some cases, often alongside tobacco chewing.

VC is characterized by a papillary, well-differentiated epithelial proliferation with minimal dysplasia. Our cases frequently showed verrucous hyperplasia with minimal nuclear atypia and a pushing rather than infiltrative growth pattern, which aligns with previous histopathological descriptions. A study by Kraus and Perezmesa (1966) emphasized that VC lacks the usual features of SCC, such as nuclear pleomorphism and increased mitotic activity, making it distinct in its behavior and response to treatment[23].

Radiographically, 9 cases (14.8%) in our study showed evidence of bone invasion, reinforcing findings by Shear and Pindborg (1980), who reported that VC can cause underlying bone destruction in advanced cases, despite its slow-growing nature[24].A study published by Bansal et Al noted that while VC is generally a benign lesion with minimal aggressive potential, long- standing cases can show transformation into squamous cell carcinoma[25]. Additionally, a case report *samira et Al* discussed squamous cell carcinoma arising in a multiple verrucous epidermal nevus, emphasizing the importance of monitoring for malignant transformation in such lesions[26].These studies suggest that while VC typically remains confined to the epithelium, there is a potential for transformation into more invasive forms like SCC, underscoring the need for careful monitoring and management.

In our study , metastasis was observed in 3 cases (4.9%), while extranodal extension (ENE) was also noted in 3 cases (4.9%). Historically, VC has been considered non-metastatic or minimally metastatic. Ackerman initially classified VC as a locally aggressive but non-metastasizing tumor, an assertion supported by many subsequent studies. However, some cases of lymph node involvement and distant metastasis have been reported in more recent studies. For instance, a case report discussed a 67-year-old male patient with hybrid verrucous carcinoma exhibiting bone invasion and bilateral lymph node metastasis, despite the absence of a clear SCC

component.

This suggests that even minimal invasive characteristics within VC can lead to metastatic behaviour[27].

Another study highlighted that hybrid verrucous carcinoma, characterized by the coexistence of verrucous and conventional SCC histological features, can exhibit more aggressive behaviour, including lymph node metastasis[28].

This underscores the importance of thorough histopathological evaluation to identify any conventional SCC areas within VC, as their presence may necessitate more aggressive management. These findings emphasize the need for careful assessment of VC cases, particularly those with hybrid histology, to determine the appropriate therapeutic approach and predict potential metastatic behavior.

VC is typically a slow-growing, well-differentiated tumor with a low propensity for metastasis. In contrast, hybrid tumors—those exhibiting both VC and conventional squamous cell carcinoma (SCC) histological features—may demonstrate more aggressive behavior, including lymphatic spread. *Another* case report by Cunha et Al of VC with foci of invasive SCC, highlighting the potential for increased metastatic risk in such hybrid lesions[29].

Additionally, a case report described a rare instance of VC of the scalp, noting that while VC typically has low metastatic potential, certain factors may contribute to a more aggressive course[30].

Our findings align with this, as metastatic cases in our cohort were among patients with longstanding habits. Similarly, Shafer et al. (1975) documented ENE in only 2-5% of cases, comparable to our study's 4.9% incidence.These findings underscore the importance of thorough histopathological evaluation to identify any conventional SCC areas within VC, as their presence may necessitate more aggressive management[31].

Another OSCC study found no significant correlation between bone invasion and nodal metastasis, instead highlighting that the worst pattern of invasion (WPOI-4,5) was a stronger predictor of nodal spread. In contrast, the VC dataset reported nodal metastasis in only 3 cases (4.9%), reinforcing the well-documented low metastatic potential of VC. This comparison suggests that while OSCC metastasis is influenced by invasive tumor patterns, VC rarely metastasizes, further distinguishing their biological behavior[32].

One study found a strong correlation between high METTL5 expression and nodal metastasis in OSCC, indicating its role in tumor progression and poor prognosis. In contrast, the VC study reported nodal metastasis in only 3 cases (4.9%), reinforcing its low metastatic potential. This comparison highlights that while OSCC frequently exhibits nodal spread influenced by molecular factors like METTL5, VC remains largely localized with minimal lymphatic involvement[33].

VC is often diagnosed at an early stage (T1 or T2), as it presents as a slow-growing exophytic lesion. However, some cases in our study exhibited advanced-stage disease with T4 classification. Studies by van der Waal et al. (2011) suggested that delayed diagnosis, particularly in habitual tobacco users, often results in T3 or T4 staging at presentation. This emphasizes the importance of early detection and regular screening in high-risk populations[32].

Prognosis of VC varies with the stage at diagnosis. National survey of head and neck verrucous carcinoma reported that survival rates significantly decreased from 86.7% for Stage I to 46.8% for Stage IV, emphasizing the importance of early detection and treatment[33]. Additionally, Kademani et al. (2005) conducted a retrospective study on intraoral squamous cell carcinoma, identifying stage and grade as significant prognostic factors. While this study focused on squamous cell carcinoma rather than VC, it highlights the impact of disease stage on patient outcomes[34].

These findings underscore the critical role of early diagnosis and intervention in improving survival rates for patients with verrucous carcinoma.This is particularly relevant to our dataset, where a small subset of cases showed aggressive behavior, correlating with more advanced staging.

The primary treatment modality for VC is surgical excision with adequate margins, as the tumor has a low propensity for distant metastasis. In our cohort, all cases underwent surgical resection, consistent with treatment guidelines[35]. According to a study by Batsakis (1999), radiotherapy is not the preferred treatment due to concerns of anaplastic transformation, where VC may undergo dedifferentiation into a more aggressive SCC [36]. Radiation therapy for VC has been associated with potential malignant transformation. A study by Perez et al. discussed cases where VC underwent anaplastic transformation after radiation treatment, suggesting that ionizing radiation might contribute to this change [37].

The role of adjuvant therapy in VC management is typically reserved for specific scenarios. A retrospective review by Franklyn et al. (2017) indicated that adjuvant therapy is considered in cases with close resection margins, nodal involvement, or recurrent disease. Their findings showed that most patients were effectively managed with surgery alone, with adjuvant therapy administered selectively [38].

These studies underscore the importance of individualized treatment planning in VC, highlighting the potential risks associated with radiation therapy and

the selective use of adjuvant treatments based on specific pathological features.Our findings are consistent with this, as only a few cases required additional treatment beyond surgery.

VC has a relatively good prognosis, with low recurrence rates following complete excision. However, recurrence rates vary based on factors such as tumor size, presence of dysplasia, and margin status. Achieving clear surgical margins is crucial in reducing the recurrence of VC. Incomplete excision has been associated with higher recurrence rates. A study highlighted that VC has a high recurrence rate if incompletely excised, and recurrent tumors can be more aggressive than the original, with potential bone and cartilage invasion [39]. Another study found Verrucous carcinoma, though less metastatic than conventional SCC, poses clinical challenges due to its potential transformation and involvement of molecular regulators like miR-21 in tumour progression [40].

While VC is typically characterized by local invasion with a low potential for metastasis, certain cases exhibit more aggressive behaviour. A case report described a 67-year-old male patient with VC causing bone invasion and bilateral lymph node metastasis, despite the absence of a clear squamous cell carcinoma component. This suggests that even minimal invasive characteristics within VC can lead to metastatic behaviour.

In our dataset, follow-up details were not available, but the presence of invasion and metastasis in a subset of cases suggests the potential for recurrence in high-risk patients.

OSCC in younger patients exhibits aggressive features such as high nuclear pleomorphism, tumor budding, and a stroma-rich microenvironment, suggesting a more invasive phenotype. In our study VC, though slow-growing, has the potential to transform into invasive SCC, indicating that prolonged epithelial instability may contribute to malignant progression. Both conditions highlight the role of the tumor microenvironment in dictating behavior, with OSCC showing early aggressive changes and VC demonstrating a gradual transformation risk [41-44].

CONCLUSION

Our study highlights key clinical and pathological features of VC, aligning with findings from previous research. The male predominance, strong association with tobacco-related habits, low metastatic potential, and primary surgical treatment outcomes are consistent with existing literature. However, a small subset of cases exhibited invasive behaviour, lymph node involvement, and extranodal extension, emphasizing the need for early diagnosis and close monitoring of high-risk patients. Future studies should focus on molecular and genetic markers to further understand the progression of VC and improve targeted treatment strategies.

DECLARATIONS

Ethical statement

This study was performed in line with the principles of the Declaration of Helsinki.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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RESEARCH ARTICLE

RADIATION AWARENESS AMONG DENTAL PRACTITIONERS IN WESTERN MAHARASHTRA: A CROSS-SECTIONAL STUDY

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ABSTRACT

Objective: This study aimed to evaluate the awareness and implementation of radiation protection measures among dental practitioners in Western Maharashtra, with a focus on knowledge, attitudes, and clinical practices related to radiographic safety.

Materials and Methods:A descriptive cross-sectional survey was conducted among 200 dental practitioners using a structured questionnaire comprising 10 key questions. Descriptive statistical analysis was done by using SPSS (version 23).

Results: The majority of participants demonstrated awareness of radiation safety, with 100% performing clinical evaluations before radiographic prescriptions and 87% obtaining informed consent. However, only 74% explained radiation risks to patients, and just 28% used film-holding devices, highlighting gaps in protective practices. 73% of participants stated using TLD badges. Notably, 98% stood behind a protective shield during exposure.

Conclusion: Although the findings indicate a generally positive trend in radiation awareness among dental practitioners, inconsistencies persist in the implementation of optimal safety practices, particularly in areas such as informed consent, use of film holders, and radiation dose monitoring. These gaps underscore the need for enhanced continuing dental education, stricter adherence to safety protocols, and reinforcement of regulatory guidelines to ensure the safe use of radiography in clinical settings.

Keywords*:* Dental radiography, radiation safety, ALARA principle, informed consent, lead apron, TLD badge, Western Maharashtra, dental practitioners, radiation protection practices.

INTRODUCTION

Radiographic imaging has become an indispensable tool in modern dentistry, facilitating accurate diagnosis and effective treatment planning. Dental X-rays enable clinicians to detect conditions not visible during routine examinations, such as interproximal caries, periapical lesions, and bone pathologies, thereby enhancing patient care[1].

Despite the diagnostic benefits, dental radiography involves exposure to ionizing radiation, which, albeit low in individual doses, can accumulate over time and pose

health risks to both patients and practitioners[2]. Potential risks include cellular damage and an increased likelihood of malignancies, particularly with repeated exposures [3]. Therefore, it is imperative to balance the diagnostic advantages of radiographs with the principles of radiation protection.

A fundamental concept in radiation safety is the ALARA principle-"As Low As Reasonably Achievable." This principle advocates for minimizing radiation exposure by employing the lowest possible dose to achieve the necessary diagnostic outcome[4]. Implementing ALARA involves using appropriate shielding, selecting suitable imaging techniques, and limiting the frequency of radiographic examinations.

Best practices in dental radiography encompass several strategies aimed at reducing radiation exposure. These include the use of digital radiography, which significantly lowers radiation doses compared to traditional film-based methods[5]. Additionally, employing rectangular collimation restricts the X-ray beam to the area of interest, thereby minimizing exposure to surrounding tissues [6]. Regular maintenance and calibration of radiographic equipment are also crucial to ensure optimal performance and safety [7].

Educational training plays an essential role in encouraging radiation safety. Dental professionals must be well-versed in radiographic techniques, radiation biology, and protection measures. Continuous professional development programs and adherence to updated guidelines are essential to maintain high standards of practice [8].

Effective communication with patients regarding the necessity and safety of radiographic procedures is equally important. Providing clear explanations about the benefits and potential risks fosters trust and facilitates informed decision-making. Obtaining informed consent before radiographic examinations is not only an ethical obligation but also a legal requirement in many jurisdictions [9].

Regulatory bodies, Atomic Energy Regulatory Board (AERB) in India, form guidelines to safeguard the use of ionizing radiation in medical and dental practices. Compliance with these regulations is mandatory and underscores the ethical responsibility of dental practitioners to safeguard patient and staff well-being [10]. Despite the availability of guidelines and training resources, studies have indicated variability in the awareness and implementation of radiation safety measures among dental professionals[11].

Factors contributing to this inconsistency include a lack of access to continuing education, inadequate emphasis on radiation protection in dental curricula, and limited enforcement of regulatory standards.

In the context of Western Maharashtra, there is a paucity of data assessing the knowledge, attitudes, and practices related to radiation safety among dental practitioners. The purpose of this study was to fill this gap by evaluating the current state of radiation awareness and protective practices in the region. The findings are expected to inform targeted interventions to enhance radiation safety in dental settings.

MATERIAL AND METHOD

Study Design

A descriptive cross-sectional study was conducted over six months, targeting dental practitioners across Western Maharashtra. A total of 200 practitioners participated, selected through convenience sampling.

Data Collection Tool

A structured questionnaire comprising 10 predetermined questions was employed. The questionnaire assessed:

- Clinical evaluation before prescribing radiographs
- Patient inquiries about radiation safety
- Explanation of radiation risks/benefits to patients
- Obtaining informed consent
- Methods of holding image receptors
- Awareness of collimators
- Use of protective gear (lead aprons, thyroid collars)
- Practitioner positioning during exposure
- Maintenance and periodic checks of radiographic equipment

Data Analysis

Responses were analyzed using SPSS version 23. Descriptive statistics were computed.

Ethical Considerations

The Ethical clearance was obtained from the Ethical Committee of Krishna Vishwa Vidyapeeth (KVV), Karad, with Protocol number (123/2024-2025, dated 28/08/2024).
Informed consent was obtained from all participants. Confidentiality was maintained throughout the study.

Table 1. Summary of Survey Responses

Question	Yes (%)	No (%)
Clinical examination and history before the radiograph	100	0
Informed consent obtained	87	13
Patients ask about radiation safety	69	31
Explain the radiation risk/benefit	74	26
Sensor held by the patient	25	—
Sensor held by the operator	47	—
Sensor held using the film-holding device	28	—
Cone length used: 8 inches	67	—
Cone length used: 16 inches	33	—
Stand behind the lead shield	98	2
Use of lead apron and thyroid collar	89	11
Stand away during radiographic exposure	90	10
Use of TLD badge	73	27

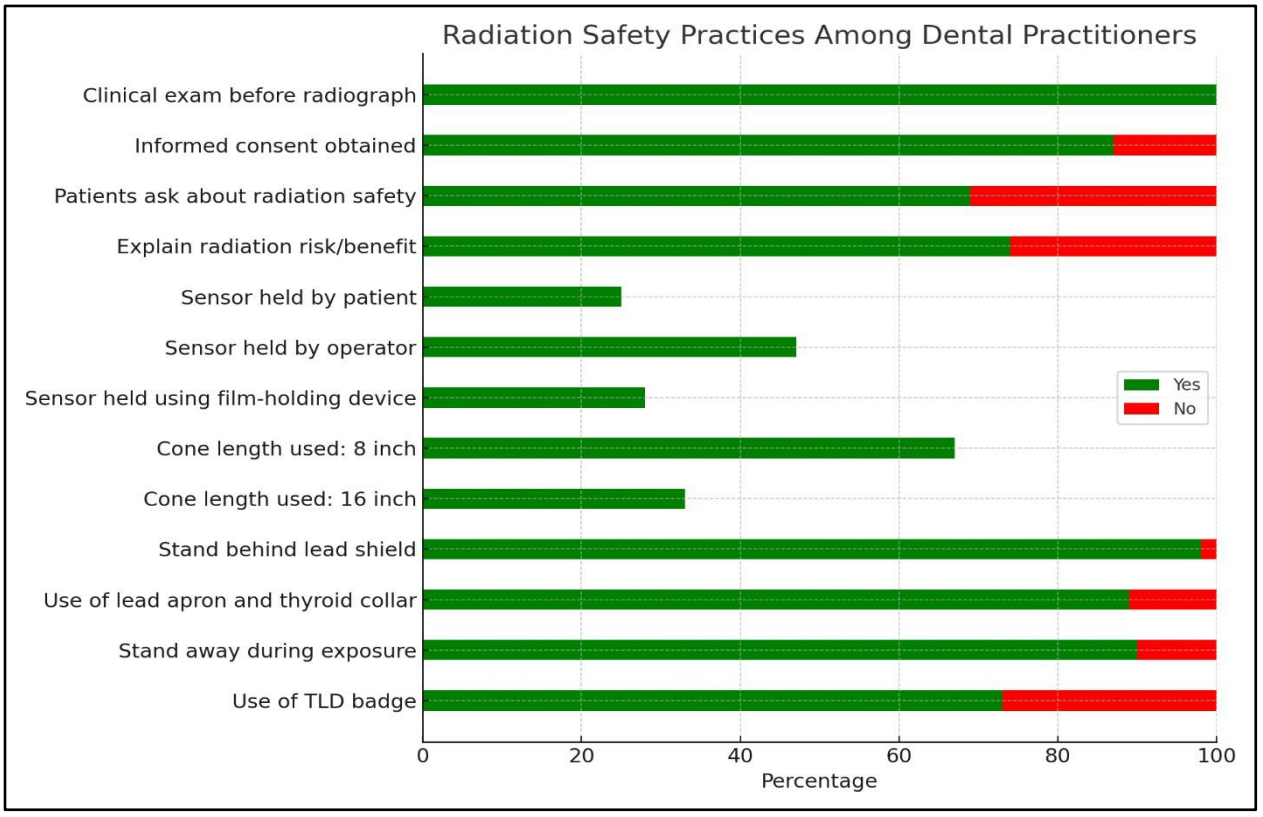


Figure 1. Radiation safety practice among dental practitioners

Key Findings:

- **Clinical Evaluation:** 85% reported conducting a thorough clinical examination before prescribing radiographs.
- **Patient Communication:** Only 40% routinely explained radiation risks and benefits to patients.
- **Informed Consent:** 35% obtained informed consent before radiographic procedures.
- **Protective Measures:** While 68% used lead aprons, only 43% employed thyroid collars consistently.
- **Practitioner Safety:** 59% stood behind a protective barrier during exposure; 23% maintained a 6-foot distance without a barrier.
- **Equipment Maintenance:** 47% conducted periodic checks of their radiographic equipment.

DISCUSSION

The study reveals a moderate level of awareness regarding radiation safety among dental practitioners in Western Maharashtra. While most practitioners recognize the importance of clinical evaluation before radiography, there is a noticeable gap in patient communication and informed consent practices.

A significant positive finding in this study is that 100% of the respondents reported considering clinical examination before radiographic exposure. This adherence to the principle of clinical justification is crucial, as it ensures that radiographs are only taken when medically necessary. The adherence to the ALARA (As Low As Reasonably Achievable) principle is encouraging, as it helps minimize unnecessary radiation exposure to both patients and operators[12]. However, amenability with ALARA includes more than just evaluating the necessity of radiographs. Optimizing radiation doses requires the proper selection of exposure settings and the application of effective shielding methods. Studies have shown that while clinical justification is important, it must be accompanied by technical measures like adjusting exposure parameters (e.g., exposure time, tube potential, and current) and using appropriate shielding techniques to achieve dose optimization[13]. Without these considerations, the mere evaluation of necessity may not fully reduce radiation exposure.

The findings reveal that 87% of the respondents reported taking informed consent from patients, and 74% explained the potential risks associated with radiation. These practices align with legal and ethical standards in patient care [14]. Transparent communication is essential for fostering trust between the dentist and the patient, which in turn improves the overall patient experience. Furthermore, according to the American Dental Association, informed consent not only serves as a legal safeguard but also empowers patients to make well-informed decisions regarding their treatment options [15]. Despite this, the fact that a portion of practitioners still fail to adequately explain radiation risks highlights a gap in ethical communication. This deficiency could lead to patients feeling uninformed or uncertain about the procedures, which may lead to legal implications and undermine the patient's autonomy. Thus, enhancing communication and ensuring that all patients receive clear, detailed explanations of the risks and benefits of radiographic procedures is crucial.

The study found that 89% of dental practitioners used lead aprons and thyroid collars during radiographic procedures, which aligns well with international guidelines on radiation protection [16]. These protective measures are effective in reducing unnecessary radiation exposure, particularly in sensitive areas like the thyroid and reproductive organs. However, only 28% of respondents used film-holding devices, which play a critical role in reducing radiation exposure to the operator. Operator-held radiographs, reported at 47%, are contrary to recommended safety practices. Holding films manually increases the risk of occupational radiation exposure to dental staff [17].

The study revealed that most practitioners used an 8-inch cone, which is associated with higher levels of scatter radiation compared to a 16-inch cone. Research has shown that the use of a longer cone (16 inches) helps to reduce the amount of scatter radiation, thereby decreasing the overall radiation dose to the patient and improving image quality [18]. The 16-inch cone allows for a more parallel beam of radiation, which leads to better collimation, further optimizing the radiographic procedure by reducing the area exposed to radiation. Although shorter cones are easier to handle, they should be replaced by longer cones where feasible to adhere to best practices in radiographic safety.

The study also highlighted that 98% of dental practitioners adhered to standing behind lead shields during radiographic exposure, and 90% maintained an appropriate distance from the radiation source. These measures align with established protocols for protecting dental staff from unnecessary radiation exposure [19]. However, the use of thermoluminescent dosimeter (TLD) badges for personal radiation monitoring was reported by only 73% of participants. TLD badges are essential tools for monitoring and recording the cumulative radiation dose received by personnel, which is vital for ensuring that occupational exposure stays within a safe limit [20]. The underuse of TLD badges suggests that more awareness and training are needed to emphasize their importance in protecting the health of dental professionals.

Variability in radiation safety practices, including inconsistent use of collimators, sensor handling, and informed consent procedures, underscores the need for structured continuing dental education (CDE) programs. While the majority of practitioners seem aware of the basic radiation safety principles, there is still a significant gap in the consistent application ofthese practices. Research has indicated that regular participation in CDE programs is associated with improved knowledge and adherence to radiographic safety protocols [21]. Continuing education programs can provide updates on the latest guidelines, reinforce the

importance of radiation safety, and address areas where dental practitioners may be lacking in their practice.

CONCLUSION

In conclusion, while the study reveals positive trends in radiation safety practices among dental practitioners in Western Maharashtra, several areas require attention to further enhance safety for both patients and dental professionals. Improvements are needed in areas such as the consistent use of film-holding devices, informed consent procedures, and radiation dose monitoring for personnel. By addressing these gaps through better training, stricter adherence to guidelines, and the use of modern protective equipment, dental practitioners can ensure safer radiographic practices, which will benefit both the patients they treat and the staff who operate the equipment. While these findings are meaningful, they should be interpreted in light of the study's limitations, including its reliance on self-reported data, limited geographic scope, lack of observational validation, and exclusion of patient perspectives.

Future studies with broader regional coverage, objective assessments of clinical practices, and longitudinal follow-up are recommended. Additionally, greater emphasis on structured continuing education and hands-on training programs can help bridge the gap between knowledge and consistent, safe practice. By addressing these limitations, future research can contribute more comprehensively to the advancement of radiation safety in dental practice.

DECLARATIONS

Ethical statement

This study was performed in line with the principles of the Declaration of Helsinki.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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RESEARCH ARTICLE

KNOWLEDGE, ATTITUDE AND PRACTICE OF GENERIC MEDICINES AMONG DENTAL GRADUATES AND POSTGRADUATE STUDENTS OF NORTH MAHARASHTRA REGION.

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ABSTRACT

Background: Rising healthcare costs are a global concern, especially in developing countries where nearly 80% of healthcare expenses are paid out-of-pocket. A major contributor to this expenditure is the cost of medications. Generic medicines, which are therapeutically equivalent to branded drugs and significantly more affordable, offer a practical means to reduce healthcare costs while maintaining treatment standards. Despite India being a leading producer of generic drugs, their prescription—particularly in dentistry—remains underutilized.
Objective: To evaluate and compare the knowledge, attitude, and practice (KAP) of dental students regarding generic medicine prescription.
Method:A cross-sectional survey was conducted in December 2019 among 100 participants (22 MDS students and 78 interns) at the School of Dental Sciences, Karad, Maharashtra. A self-administered, pre-validated questionnaire with 15 items was used to collect data on demographics and KAP related to generic medications. Responses were recorded in dichotomous format (Yes/No), and one open-ended question sought suggestions for promoting generic use. Data were analyzed using Microsoft Excel and MedCalc software; the chi-square test was applied for statistical comparison.
Result: Participants had a mean age of 23.14 years; 76% were female. Only 52.4% knew generics share the same composition and dosage as branded drugs, and 31% knew repeated clinical trials are not required for generics. About 31% believed branded medicines are more effective, and 54.8% felt generics are often manufactured in substandard facilities. Just 47.6% reported prescribing generics, and 69% had never read comparative studies between branded and generic drugs. The most influential factors in prescribing decisions were patients' economic status (85.7%) and drug efficacy/safety (83.3%). Most participants supported implementing awareness workshops, educational training, and stricter regulations to improve generic prescribing habits.
Conclusion: Despite policy efforts, the prescription of generics among dental professionals is limited due to knowledge deficits and ongoing misconceptions. Targeted educational programs, mandatory training, and regulatory reinforcements are necessary to foster confidence in generic medications. Expanding this research across institutions will offer more generalized insights and support national strategies to enhance access to affordable healthcare through rational prescribing.

Keywords*:* generic medicines, educational programs, dental professionals, dental graduates, postgraduate students

1.INTRODUCTION

The increasing health-care expenditures remain a serious concern for the health-care system worldwide. In developing countries approximately 80% healthcare expenditure is paid from patients' pocket.[1] As patient is bearing the cost of treatment themselves, it is necessary to keep health-care expenditure minimal without compromising the quality

of care. One of the major components of health-care expenditure is cost of the medicines.[2] The price of generic medicines is 10-80% lower than their branded equivalents. Hence, the usage of generic medicines can improve the access to health-care without conceding the quality.[3]Generic medications are substitutes for original, branded medications that are just as safe, effective, and of comparable quality. In clinical practice, prescribing generic medications can drastically reduce health care costs. This can also assist government organizations and health care payers[4]. Increasing the use of generics can assist provide high-quality healthcare to the vast population of India, given the country's limited resources. This will increase the population's capacity to afford high-quality healthcare services[5].

Various stakeholders in the Indian context have made a number of steps to promote the use of generic prescriptions in clinical settings. In 2018, the Medical Council of India revised its code of conduct for physicians, advising all doctors to prescribe medications with generic names[5]. This program's objective is to provide high-quality, unbranded medications reasonably priced and accessible to the nation's impoverished citizens. Even though there were 3200 Jan Aushadi outlets open in India as of 2018, this was still insufficient to satisfy the needs of the Indian populace[6].

In the Indian context, generic prescription is not widely used, despite all the efforts. Branded medications account for 90% of the domestic pharmaceutical business in India, which is worth at least Rs. 1,000,000 crore[7]. It is evident that there are not enough generic name counterparts of branded medications available in the Indian market, even though India is one of the world's largest producers and exporters of generic medications[8]. Numerous studies have demonstrated that one of the main barriers to the adoption of generic prescribing is the way doctors see generic medications. [9, 10]

Additionally, it has been noted that one of the obstacles to the adoption of generic prescription is a lack of knowledge regarding the regulatory requirements utilized in generic manufacture. [11]. One of the main obstacles to prescribing generics is a lack of understanding about them. This study aims to evaluate dental professionals' knowledge about generic prescription.

Aim

To compare the knowledge, attitude, and practice of dental students on inexpensive prescribing.

METHODS

In December 2019, a cross-sectional survey was conducted among postgraduate (MDS) dentistry students and interns. After receiving approval from the Institutional Ethics Committee (136/2024-2025), the study was carried out at the School of Dental Sciences, Karad, Maharashtra. The survey comprises self-administered, pre-validated questions relevant to the Indian context. These inquiries were drawn from published works about Indian setup. [12–15] The questionnaire included 15 questions regarding participants' demographic information as well as their knowledge, attitude, and practice (KAP) of generic medications.

Ten questions on generic medicine knowledge, five questions about participants' attitudes toward generic medicine, six questions about generic medication practice, and one question asking for recommendations to enhance generic prescribing in India were all included in the questionnaire. Dichotomous replies (yes or no) were used to gather the responses. There was just one open-ended question asking for recommendations on how to enhance generic prescription in India. Ten institute interns were chosen at random to participate in the questionnaire's preliminary testing.

The purpose of the literature search was to locate studies evaluating dental practitioners' knowledge of generic medications in an Indian context. Data was gathered and analyzed using microsoft excel. The Med Calc statistical program was used to analyse the data collected from dentists. [16] The Chi-squared test was used to compare the responses from the two investigations.

RESULTS

Of the 100 participants in this study, 22 were MDS students and 78 were interns. Participants are 23.14 years old on average. Of the participants, 24% were men and 76% were women.

Knowledge

The knowledge related questions and frequency of response of the participants is presented in Table 1.
The chart shows that 61.9% of dentists concurred that generic medications were meant to be used in place of branded ones.

Just 50% of participants knew that generic medications were available on the market after the innovator drug's patent had expired, and 52.4% of dentists were aware that the composition, dosage, and indications of generic medications were identical to those of their branded equivalents. Thirty-one percent of survey participants knew that producing generic medications did not need repeating preclinical and clinical research.

Attitude

According to the data below, the majority of dentists

(76.2%) believed that generic medications did not have more adverse effects than branded ones. Additionally, 31% of participants think that brand-name products are more effective than generics. In contrast to brand-name medications, over half of participants (54.8%) thought that generic medications were frequently produced in inferior facilities.

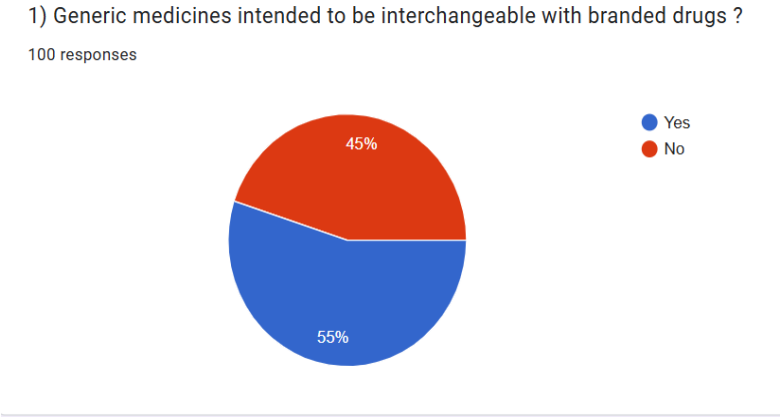
Medications. An further 50% of participants disagreed that generic medications are less expensive than name-brand ones because they are of lower quality.

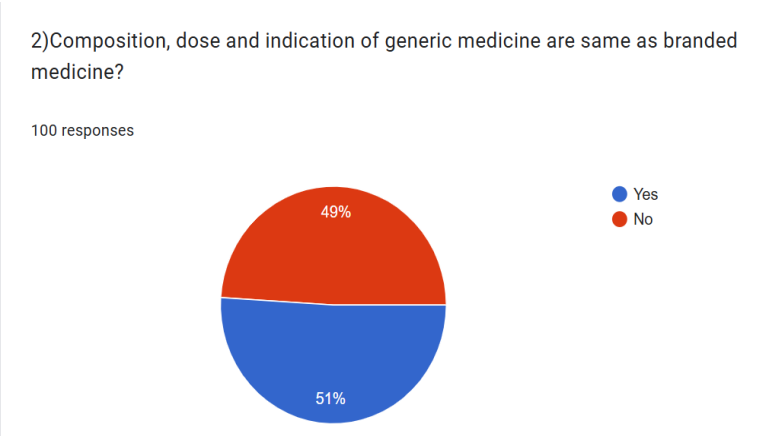
Names of medications. Of the participants, three-quarters (76.2%) thought that more rigorous quality testing of generic medications was necessary. The majority of participants (81%) said that a training program should be established to raise patients' and physicians' knowledge about generic medications.

Practice

Pie chart provides a summary of the practice-related question and the participants' answers. 47.6% of participants, or over half, admitted to prescribing generic medications. Sixty-nine percent of participants had never read an article comparing the safety and effectiveness of generic and name-brand medications. The vast majority of physicians, 45.2%, did not believe that transferring a patient from brand-name to generic medications might alter the course of treatment. An further 69% of participants expressed discomfort if the pharmacist switched the brands of medications they had been prescribed. Nearly half of respondents (54.8%) agreed that replacement should be used whenever a generic is available.

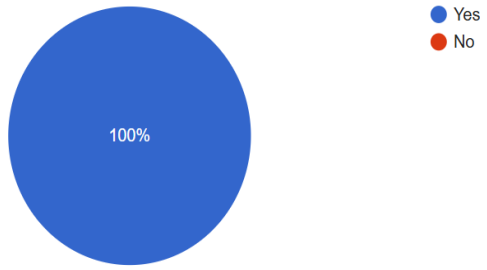
The patient's economic profile (85.7%) and the medication's effectiveness and safety profile (83.3%) were the two most important factors when prescription medication to patients. 76.2% of participants took into account the availability of medications at pharmacies, while 69% of people took the cost of medications into account. These two were the next crucial factors that determined which medication should be supplied. Just 45.2% of participants took patient demand to prescribe inexpensive medications into consideration. The participants recommended that a variety of workshops, seminars, and continuing education initiatives be used to raise awareness of generic prescription. Additionally, participants recommended that legislation pertaining to the prescription of generic medications be created and implemented.





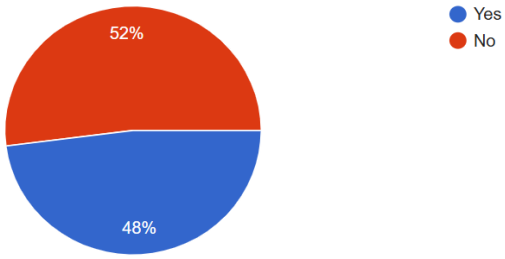
3) Bioequivalence is necessary for generic approval?

100 responses



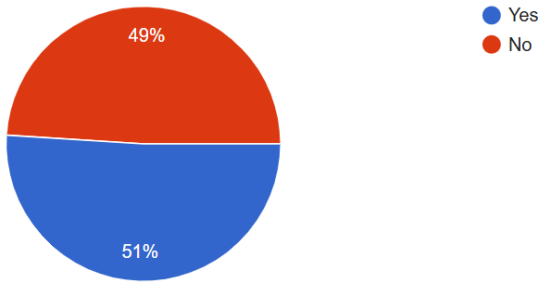
4)To reduce overall health expenditure generic medicine are important tool?

100 responses



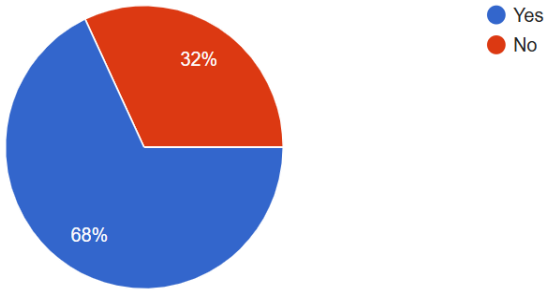
5) Brand name medicines produce lesser effect than generic medicine?

100 responses



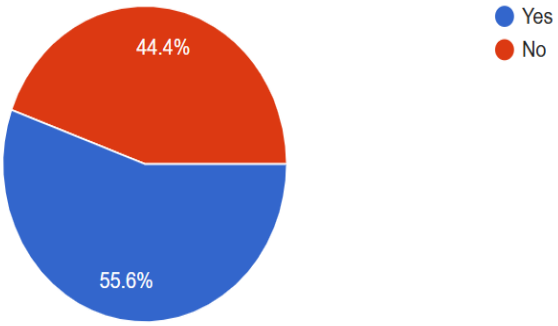
6) Generic are not as effective as brand names?

100 responses



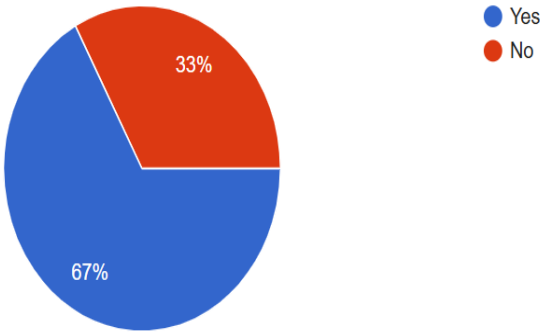
7) Brand name drugs are made in modern manufacturing facilities and generic are made in substandard facilities?

99 responses



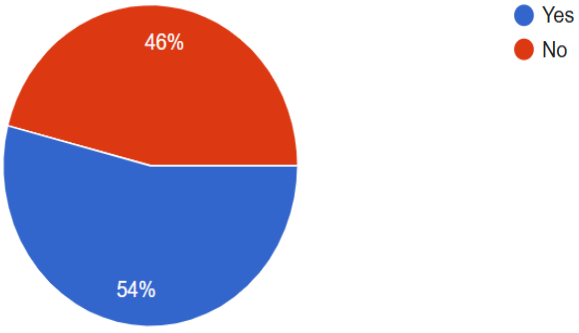
8) Generic drugs cost less because they are inferior to brand name drugs?

100 responses



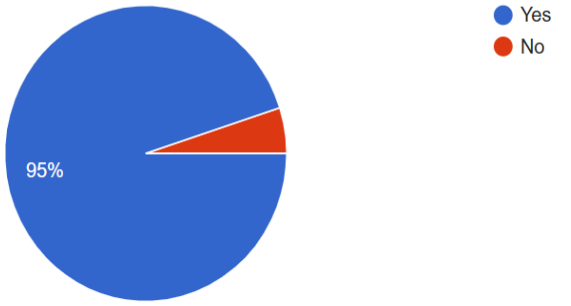
9) Quality testing of generic should be made more vigorous?

100 responses



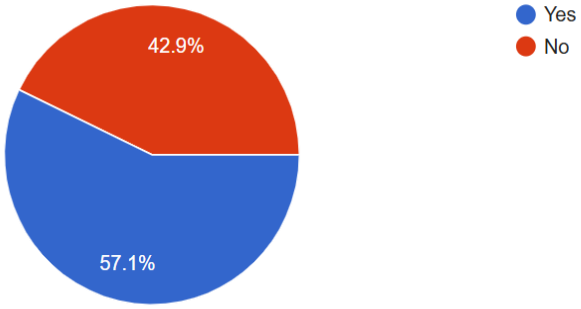
10) Do you think there should be training program to increase the awareness regarding generic drugs?

100 responses



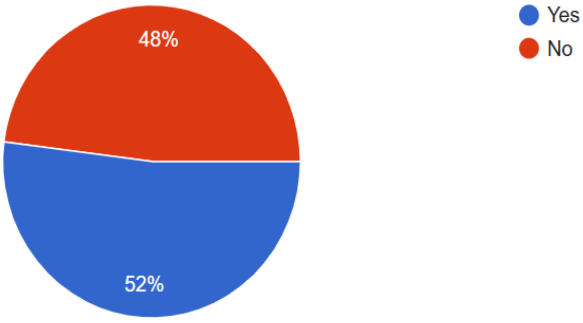
11)Pharmacist should be allowed to substitute the innovator drug with generic version?

98 responses



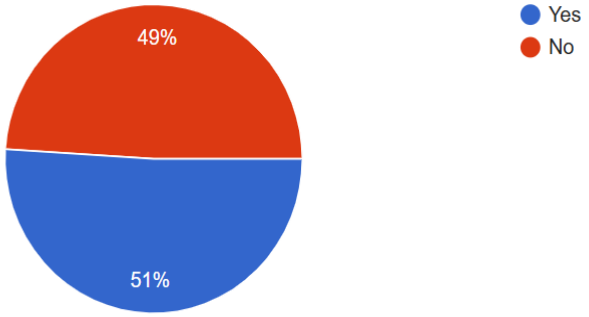
12) Do you prescribe generic medicine?

100 responses



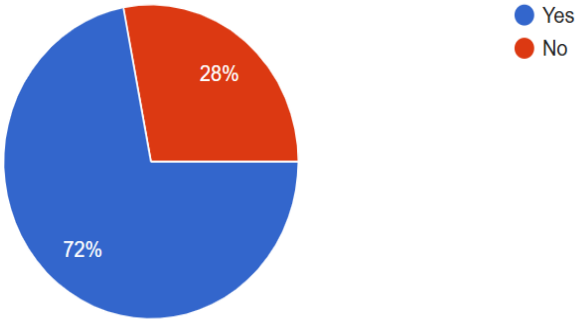
13)Have you anytime read any article on comparison of safety and efficacy of generic versus branded medicines?

100 responses



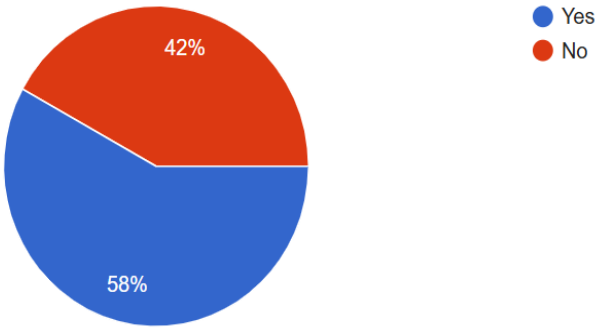
14) Do you think that switching a patient from a brand name to generics may change the outcome of the therapy?

100 responses



15) Do you support substitution in all cases where generic is available?

100 responses



DISCUSSION

To the best of the author's knowledge, this is the first study to be conducted in India that evaluates the KAP of generic medications among dental prescribers. In India, KAP of generic medications among medical practitioners has been conducted and published. According to a study, the global market for dental drugs is anticipated to grow at a compound annual growth rate (CAGR) of approximately 5.8% over the next five years, reaching 5720 million USD in 2024 from 4320 million USD in 2019.17 Similarly, the dental prescribing market in India is expanding rapidly at a rate of 8%. The market for dental prescriptions for specific disorders and oral care products is valued at nearly 550 crores INR.[18.]

All of this highlights the fact that dentists are among the main prescribers of medications so their prescription procedures need to be revised. Several nations, notably Mexico, have stated that dental professionals frequently make prescription errors due to a lack of the finest pharmacological education[19].

With a few exceptions, dental and medical professionals' perceptions and comprehension of generic prescription are often the same. Dental professionals had somewhat less understanding than medical professionals in areas including bioequivalence research, DCI regulation for prescribing generics, and the composition, dosage, and indications of generic medications. The setup discrepancies may be the cause. Typically, dentists practice in specialist clinics rather than mainstream hospitals.

The study's primary weaknesses are its small sample size and restriction to a particular institutional setup. Therefore, it is not possible to generalize the results of this study. The information provided by respondents is a major determinant of the research that use self-administered questionnaires. Even though participants were encouraged to complete the questionnaire on their own, factors including recollection bias and reciprocal impacts could not be totally removed. In order to understand the knowledge and practices of prescription writing, we advise that similar research be carried out in different regions of the nation.[20] We have just looked at how doctors view and comprehend generic medications. Adoption of generic prescription is also significantly influenced by patients' and other healthcare professionals' perceptions and comprehension.

Any policy or product's effective execution depends on the public's trust in its efficacy, safety, and quality. Numerous studies have demonstrated that one of the main obstacles to the widespread use of generic prescription has been the way doctors view these medications. According to a statewide poll conducted in Malaysia, 51.7% of doctors said that generic medications are less effective than name-brand ones, indicating that doctors have a poor opinion of them. Additionally, 74.2% of doctors said that branded medications had far superior manufacturing and quality control standards than generic ones.[9] Similar findings were revealed by another study carried out in Bosnia and Herzegovina. Sixty percent of respondents said that circumstances for generics production are worse. [10].

According to a different systematic research, how people see doctors depends on how developed the healthcare system is. Physicians in less developed healthcare systems describe significant differences in control procedures, requirements for generics' bioequivalence and manufacturer standards. [21] A research was carried out in Ireland following .The Health Act of 2013 made generic substitution lawful. Concerns with generic replacement in vulnerable patient populations, such as the elderly, the frail, those taking several medications, people with cognitive impairment, and people with anxiety, were brought to light by this study. [22]

According to a research done in India, the quality of the branded and generic products was similar across a range of tests. Contrary to quality testing, unfavorable opinions about the quality of medications, together with other factors, lead to the use of more costly medications. The choice of medications and healthcare providers within the local health system was explained and influenced by trust in the system.[23] Similar to this, a different study carried out in South Africa revealed a pronounced discrepancy between opinions and the real quality of medications, pointing to shortcomings in government campaigns to raise awareness of generic drugs, which is influencing the use of generic prescribing.[24]

Manufacturers' ads for their drugs have also been identified as a factor affecting prescription and dispensing patterns. According to a poll done in Ethiopia, 39.9% of doctors agreed or strongly agreed that their prescription practices will be influenced by drug makers' promotions and marketing. [25] One of the biggest obstacles to the adoption of generic prescription is undoubtedly the lack of awareness among medical and dental professionals as well as the unfavorable opinions of doctors toward generics.

The study results indicate a correlation between NAFLD and increasing age, with males being the most affected group. These findings are consistent with previous studies, including those by Toshihide[14], James [15], and Amedeo [16], which have demonstrated a close association between age and the development of NAFLD. Additionally, the study aligns with research by Ylse [17], which suggests that NAFLD prevalence is higher in postmenopausal

women and those with polycystic ovary syndrome (PCOS) compared to premenopausal women.

Moreover, it was observed that men under 55 years of age are more likely to develop NAFLD than women of the same age, with incidence rates increasing in women after menopause. This increase in incidence among postmenopausal women may be attributed to hormonal changes, particularly a decrease in estrogen levels after menopause. The dietary changes and elevated cholesterol levels, including high levels of low-density lipoprotein (LDL) in the blood, could rule as well [18]. Furthermore, women in menopause often experience weight gain with a redistribution of fat to visceral areas [18,19].

Based on BMI levels, the results showed significant differences ($P \leq 0.05$) between patients and healthy individuals, with patients exhibiting the highest ratio within the obese weight BMI category (90.0%). Whereas, healthy subjects predominantly fell within the normal weight BMI category and represented 70.0%. Both male and female patients had higher BMI levels compared to healthy individuals, with statistically significant differences ($P < 0.001$).

These findings are consistent with Fabbrini's work [20], in which a strong relationship between BMI and NAFLD was demonstrated. Obesity increases the risk of developing NAFLD due to an imbalance between fatty acid synthesis, oxidation, and excretion. High BMI levels can lead to metabolic disturbances, cardiovascular disease, and increased risk of mortality [21].

According to our results, the mean waist circumference was higher for patients compared to controls, with significant differences ($P < 0.05$). These findings are consistent with those of Zheng [22] and Cerbere [23], which demonstrated a close association between waist circumference and NAFLD. Additionally, the results are in good agreement with those of Bruno et al. [24], who found that postmenopausal females tend to have higher waist circumferences compared to males in the same age group. However, our findings are in contrast with Pinidiyapathirage's ones [25], who reported that men with NAFLD had higher waist circumferences compared to women.

Furthermore, our results revealed a high increase in cholesterol levels ($P < 0.05$) in patients compared to healthy controls. This elevation could be attributed to LDL-cholesterol breakdown, facilitated by the activity of the cholesterol acyl transferase enzyme. In fact, this enzyme is stimulated by the absence of insulin, and resulting in an increase absorption of cholesterol in the intestines. Additionally, the consumption of meals rich in saturated fats can contribute to elevated cholesterol levels in the body [26].

The deposition of increased cholesterol in blood vessels can lead to elevated blood pressure. Furthermore, cholesterol deposits with fatty substances may result in the formation of blood clots and arteriosclerosis due to the constriction of blood vessels and obstruction of blood flow [27]. These factors increase the risk of severe heart disease [28]. It is important to note that NAFLD is often associated with the increased mortality rate linked to heart and cardiovascular diseases [29]. Therefore, the observed elevation in cholesterol levels in NAFLD patients underscores the importance of managing cholesterol levels as part of the comprehensive care for individuals with NAFLD. This mitigates the risk of cardiovascular complications and improves overall health outcomes. However, this result is consistent with the findings of Altparmak's study, in which patients with liver disease exhibit higher cholesterol levels compared to healthy individuals [30]. Additionally, the results of Ho et al. revealed a direct relationship between increased cholesterol levels, portal phlebitis, and cirrhosis in NAFLD [31]. Furthermore, Enjoji et al. suggested treating patients with fatty liver disease by controlling dietary cholesterol intake [32]. Higher levels of triglycerides in patients were observed compared to healthy individuals, which lined with the results of the Leylabadlo and Kwon studies [33,34]. Finally, Khamseh showed a high association between triglycerides and NAFLD [35]. Here, all mentioned studies had similar findings to ours.

To investigate LDL, our results indicate a significant increase in the LDL level when the patient group is compared to healthy ones. These findings are consistent with those of Siddiqui and Tang studies, which demonstrated a significant elevation in LDL in patients compared to the control group [36,37]. Furthermore, the results revealed a significant increase in VLDL levels in patients compared to controls. These findings align with those of Adiels et al.'s study, which showed a strong association between VLDL and NAFLD [38].

Regarding HDL levels, the mean values were non-significantly lower for patients compared to healthy individuals ($p > 0.05$). These results are in agreement with those found by Mirhafez studies, which demonstrated decreased HDL levels in individuals with fatty liver metabolism compared to healthy controls [39]. HDL plays a crucial role in transporting cholesterol from the body's cells to the liver, where it is purified and eliminated as waste. This process is beneficial for the body's overall health.

However, in contrast, our present research showed that the ALT and AST levels were significantly higher in the patient group compared to the healthy one,

suggesting a direct correlation between NAFLD and these variables ($p < 0.05$). Our results corroborate those of Francque and Younossi, who found that NAFLD patients had higher ALT levels [40,41]. Prashanth et al. also discovered that steatohepatitis patients had higher ALT levels [42], and other research has shown that NAFLD patients have elevated AST levels [43,44].

Similarly, the ALP levels in patients of this investigation were relatively higher compared to healthy subjects with a small difference ($p > 0.05$). Thereby, these results are consistent with those of Bazick study, which showed an increase in ALP levels in patients with fatty liver and cirrhosis [45]. Furthermore, a current study demonstrated that ALP was used as an independent biomarker for cirrhosis of the liver and NAFLD in obese individuals [46], which was also indicated in patients with steatohepatitis [42].

The results of our study revealed a remarkable difference ($p < 0.05$) in levels of intelectin-1 between patients (1.10 ± 0.30 ng/mL) and the healthy group (0.41 ± 0.25 ng/mL). This could be an indicator of a relationship between NAFLD and the increase in intelectin-1 levels.

Yimaz et al. demonstrated similar findings suggesting that intelectin-1 serves as an independent indicator of hepatocyte hypertrophy [47]. Another supporting evidence is the results of Eisinger et al.'s study, which showed high levels of intelectin-1 in cirrhotic liver patients [48]. Hence, intelectin-1 levels may serve as a biomarker for NAFLD and could play a role in the pathophysiology of liver diseases.

5. CONCLUSION

Our findings demonstrate an increase in serum levels of intelectin-1 in patients with NAFLD. Intelectin-1 levels were significantly higher in NAFLD patients and exhibited the AUC and highest sensitivity in the ROC curve analysis. Furthermore, intelectin-1 showed a positive correlation with lipid profile and liver function parameters. Therefore, the elevation in plasma intelectin-1 levels, along with increased serum levels of total cholesterol, TG, LDL, VLDL, and liver function parameters, may serve as a potential indicator of liver insufficiency in NAFLD patients. Thereby, those can be utilized as predictors in the diagnosis of NAFLD.

DECLARATIONS

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Funding

None.

Conflict of Interest

The authors declare no known conflict for this work.

Ethical Aproval

The study received ethical clearance from the Ethics Committee at the National Centre for Training and Human Development/Baquba Teaching Hospital of the Iraqi Health, as well as from the Ethical Committee at the University of Diyala. All participants provided written informed consent before taking part.

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RESEARCH ARTICLE

KNOWLEDGE, AWARENESS, PERCEPTION OF DIGITAL DENTISTRY AMONG DENTAL STUDENTS - "A QUESTIONNAIRE STUDY"

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ABSTRACT

Background: To find gaps in dental students' education and training, it is essential to examine at their perceptions, knowledge, and awareness of digital dentistry. By examining dental students' present knowledge and awareness of digital dentistry and their perceptions of its importance and significance to their future practice, this study aims to fill this knowledge gap.

Objectives: To access the Knowledge, Attitude and Practices (KAP) Of Digital Dentistry Among Dental Students.

- **STUDY POPULATION-** Dental Students in Karad region.
- **STUDY DESIGN-** A Questionnaire Study
- **SAMPLE SIZE-** 100 convenience sampling technique
- **Inclusion Criteria:** Fourth-year and Intern dental students who were currently enrolled at the dental school were eligible to participate in the study.
- **Exclusion Criteria:** Students who did not meet the inclusion criteria or those who had not completed their fourth year of dental education were excluded from the study.
- **STUDY TOOL-** 10 set of pre tested questionnaire and demographic details.
- **DATA COLLECTION-** Prior informed consent were obtained to conduct the study from every student who were part of the study.The data collection method used in this study was an online survey using Google Forms. The survey was distributed to the participants via whats-app and was accessible through a unique link. The survey consisted of 10 multiple-choice questions. The data were collected and analyzed using Google Forms' built-in analytics tools, including descriptive statistics and visualizations, to provide a comprehensive overview of the participants' responses.

Results: Almost all participants had knowledge about concept of digital dentistry. Results showed that 65% of participants were see themselves using digital dentistry in further career. Results revealed that 71% participants considered that faster diagnosis were main advantage of digital dentistry in future career followed by significant time reduction (63%), better communication (60%), comfort (52%), improved patients experience (49%) and cost (43%) were the other advantages of digital dentistry.

Results also showed that 81% of the participated in the study were prefer RVG which is the part of digital dentistry in their dental practice. 72% participants were agreeing on that digital dentistry can improve patient's outcome in their practice.

Conclusion: The results of this study show a favourable outlook on digital dentistry and a willingness to explore new technologies in dentistry. This interest can foster an enriching learning environment, support innovative teaching strategies, and welcome upcoming dental academicians

Keywords*:* Perception, Awareness, Digital dentistry, dental

INTRODUCTION

The way dental care is organized, carried out, and administered has completely changed as a result of the broad use of digital technology in the field of dentistry. Digital dentistry has revolutionized the field of dentistry by offering more accurate, effective, and patient focused care using technologies like 3D printer, intra-oral scanner, computer-aided design and manufacture (CAD/CAM).[1] The introduction of these recent techniques is transforming the entire dentistry.[2,3] Digital technology has been used in clinical practice, but its introduction into dental education is still behind. Because of this, dental students could not be sufficiently equipped to implement this technology into their work, which could cause the gap between the requirements of the curriculum and the demands of modern dentistry.[4]

In addition, the speed at which technology is advancing in dentistry has made it essential for dentists to invest in lifetime learning and ongoing professional development. There is a lack of literature regarding dental student's knowledge of digital dentistry after getting into dental college. Given the increasing interest in technology mainly driven by industry, it is essential to explore whether dental students' knowledge and expectations about digital dentistry are present with the updated dental programs. Understanding dental students' perception and awareness of digital dentistry are vital in influencing institutional admission processes and decision-making regarding resource investment. The complicated aspects of digital dentistry, like the choice and use of digital instruments, the interpretation of digital images, and dealing with of technological problems, are, however, beyond the knowledge and abilities of dental students. Moreover, dental students may become delayed to accept digital technology if they believe it will not be relevant or significant to their future practice due to a lack of exposure to digital dentistry during their dental school.[5]

It is possible to create specialized educational courses and training programs that better prepare dental students for the demands of modern dental practice by acquiring an understanding of the needs and difficulties that arise when implementing digital dentistry.[6,7] In the end, our study will help the dental profession create a workforce that is more educated and able to deliver high-quality patient care that makes use of the latest advances in technology. Hence the present study was carried out to assess the current state of digital dentistry knowledge among dental students, evaluate their perception of its importance and relevance to their future practice, and identify areas for improvement in dental education and training programs.

METHODOLOGY

This study was a cross-sectional questionnaire study. Data was collected in July 2024. The target population for the study comprised 100 dental students were selected by convenience sampling technique of Karad city.

The questionnaire was written in English. The questionnaire comprised two sections where the first included 4 questions related to demographic data. The second section 10 questions to assess regarding the same. Demographic data includes name and email id, gender, year of pursuing BDS. Out of 10 questions, 4 questions were binomial i.e. having two options: yes or no type.

In order to attain the highest possible accuracy, participation in this study was voluntary and the questionnaire was anonymous and self-administered. The participants were informed the importance of answering the questions correctly and were assured of their confidentiality.

Approval was obtained from the Institutional Ethics Committee prior to onset of the study and after designing the protocol and questionnaire. (057/2024-2025) The respondents were informed about the purpose of the study using information sheets written in English I google form. The responses were anonymized to keep all the information confidential. The data collection method used in this study was an online survey using Google Forms. The survey was distributed to the participants via whats-app and was accessible through a unique link. The survey consisted of 10 multiple-choice questions. The data were collected and analyzed using Google Forms' built-in analytics tools, including descriptive statistics and visualizations, to provide a comprehensive overview of the participants' responses.

RESULTS

Demographic finding

Table 1 illustrates the demographic characteristics of the participants. 74% students were female while 26% were male. The findings indicate that 31% of participants were undergraduate students and 69% were Interns.

According to Graph no. 1, almost all the participants were aware about concept of digital dentistry. Graph no. 2, according to likert scale 25% were strongly agree that digital dentistry has useful applications in dental field followed by 51% agree and 18% were neutral. Graph no. 3 showed that 65% of participants were see themselves using digital dentistry in further career.

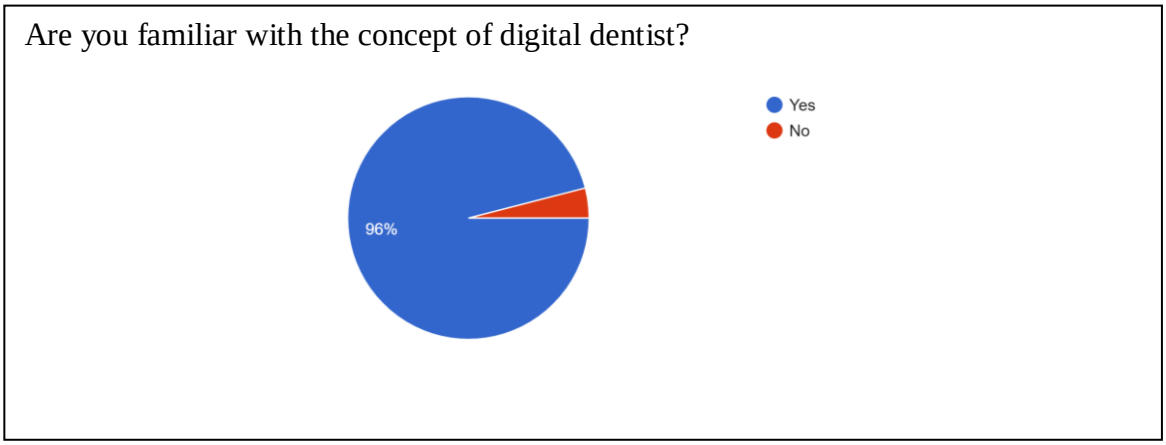
Graph no. 4 revealed that 71% participants considered that faster diagnosis were main advantage of digital dentistry in future career followed by significant time reduction (63%), better communication (60%), comfort (52%), improved patients experience (49%) and cost (43%) were the other advantages of digital dentistry.

Graph no. 5, the 98% participants think that digital dentistry provide more precise results than conventional dentistry. According to 76% of participants, digital dentistry can significantly improve the quality of dental care. Results also showed that 81% of the participated in the study were prefer RVG which is the part of digital dentistry in their dental practice. 72% participants were agreeing on that digital dentistry can improve patient's outcome in their practice. 85% students stating that conferences were the main source of getting updated to latest advances in digital dentistry followed by dental journal (43%), webinars (33%) and college training programs (23%). Making the necessary adjustments to educational resources can be beneficial.

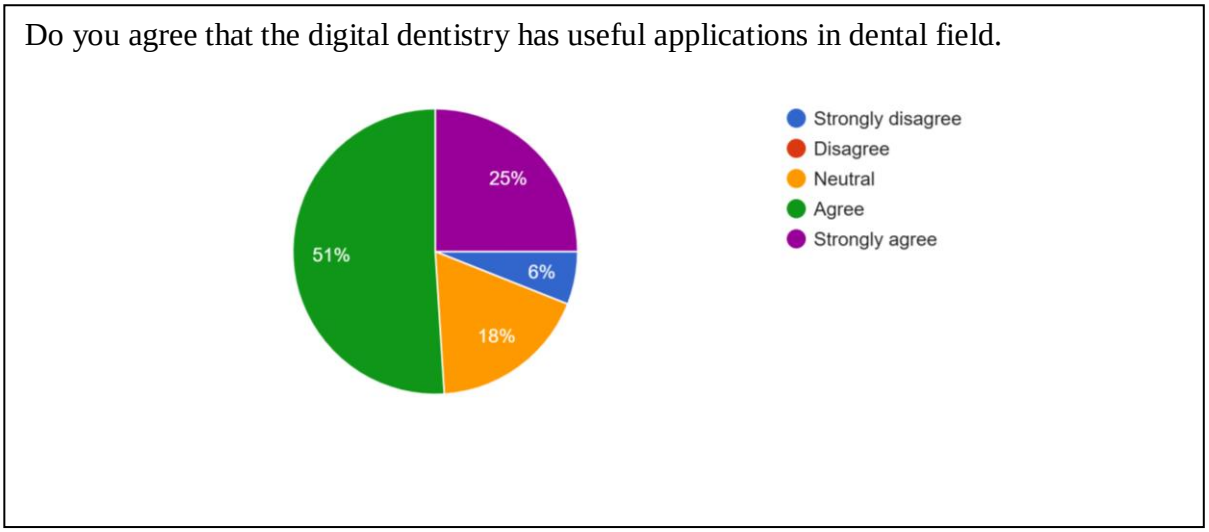
Table 1. Demographic Data of the Respondents

Demographic Factors		Frequencies (f)	Percentages (%)
Year of pursuing BDS	UG STUDENTS	31	31
	INTERNS	69	69
Gender	Female	74	74
	Male	26	26

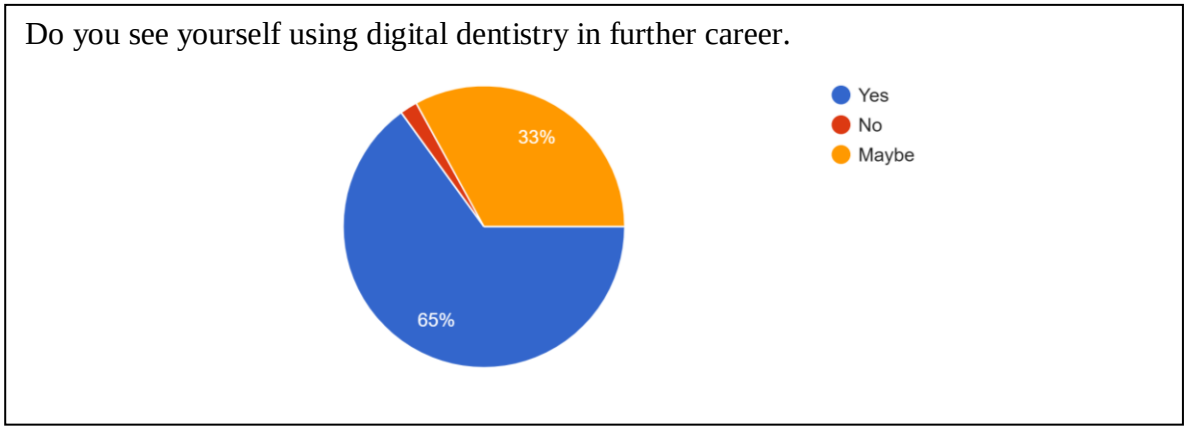
Graph N1



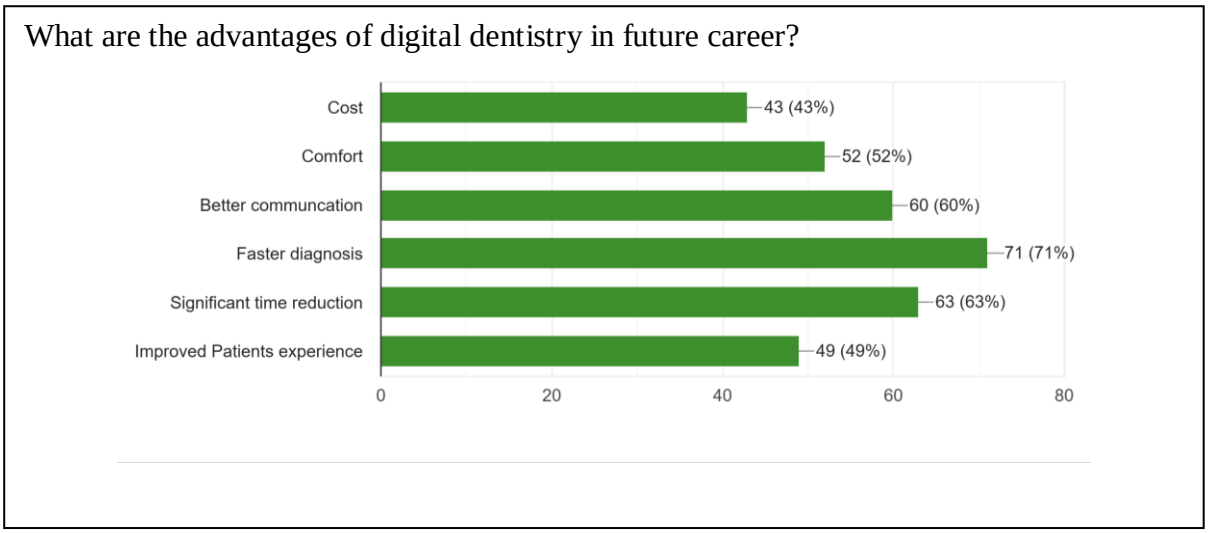
Graph N2



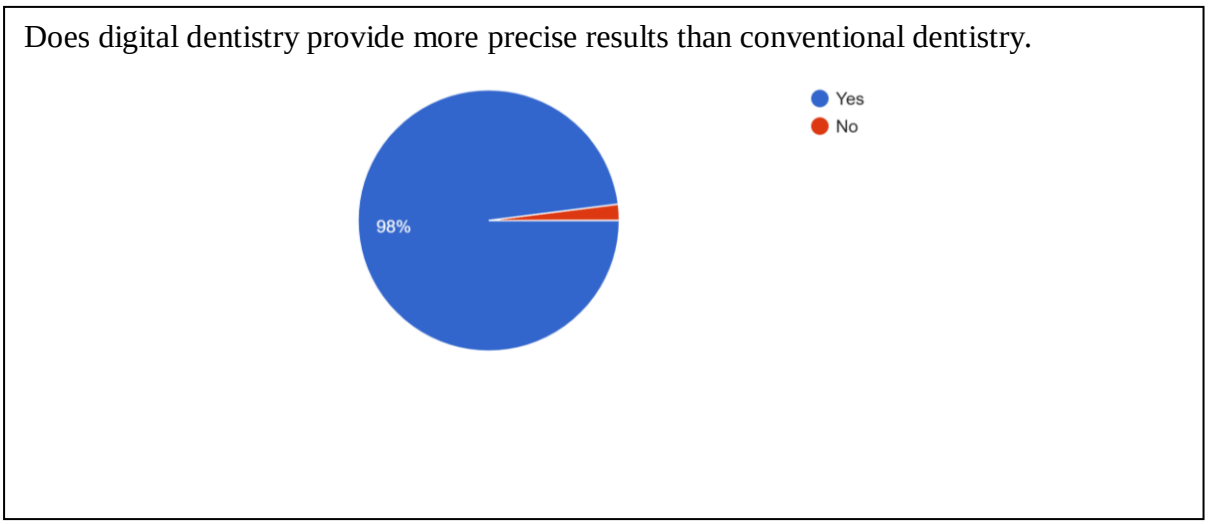
Graph N 3



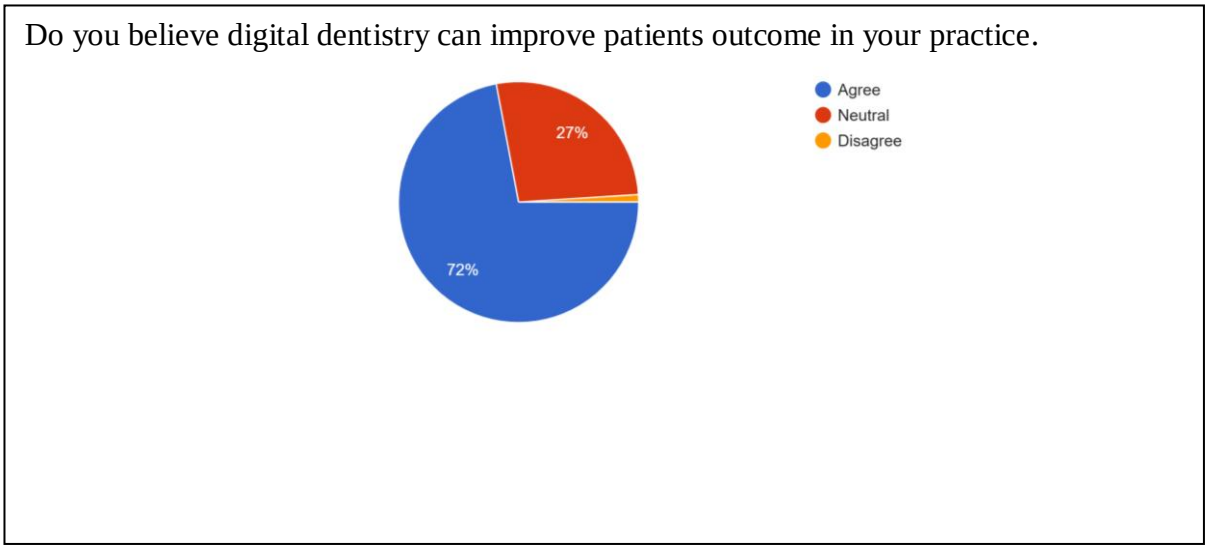
Graph N 4



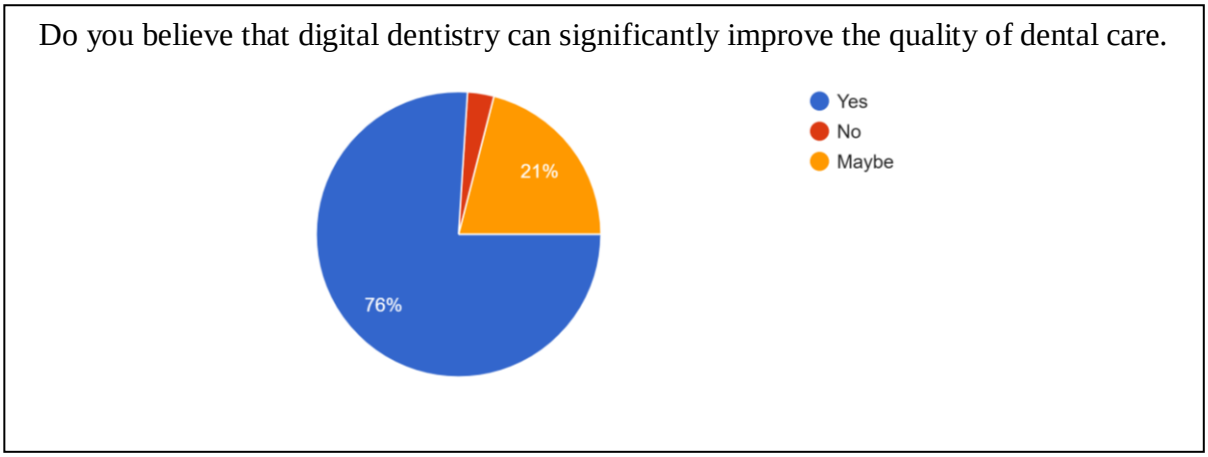
Graph N. 5



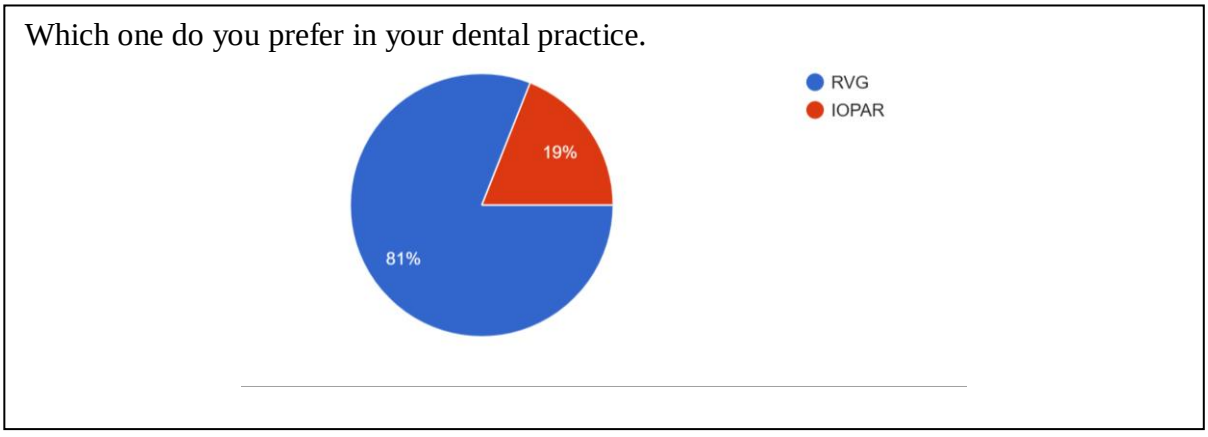
Graph N 6



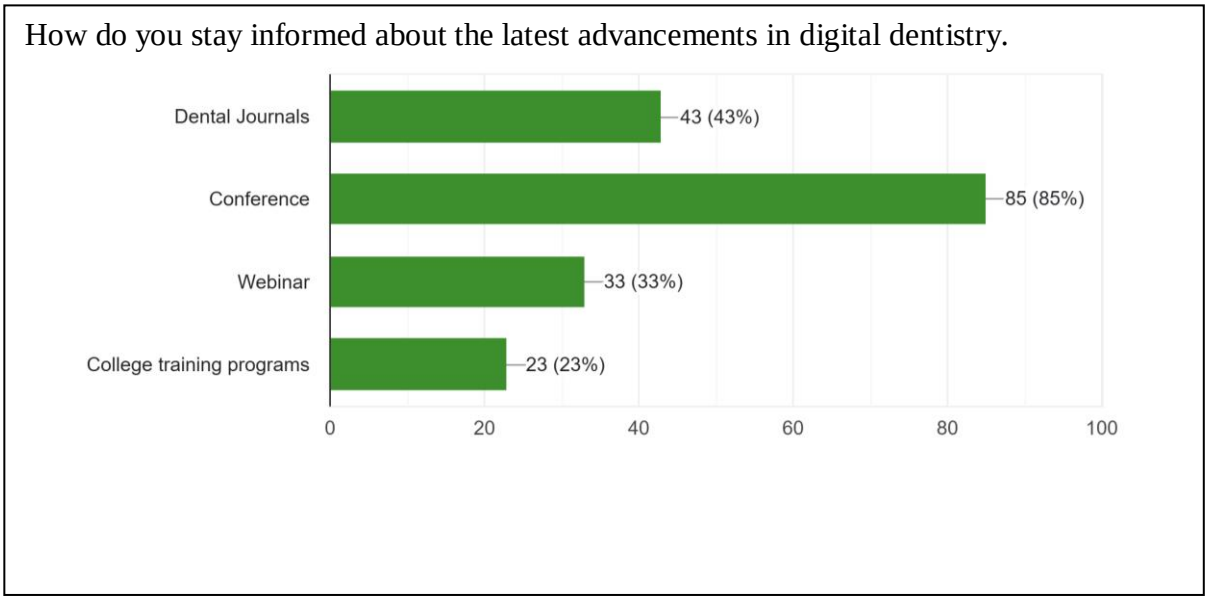
Graph N7



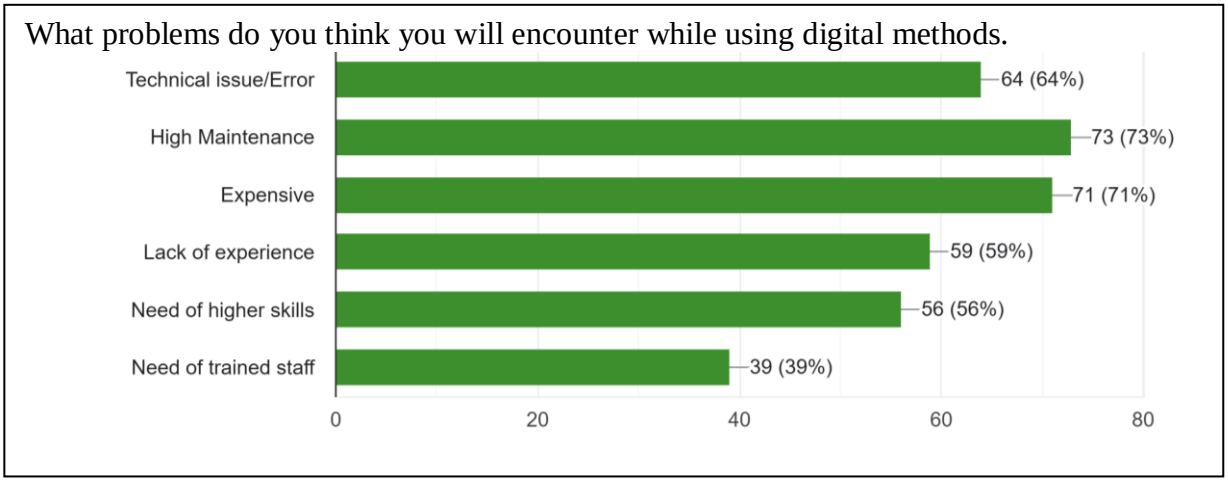
Graph N8



Graph N 9



Graph N10



DISCUSSION

As this was a cross sectional questionnaire study focuses on KAP related access the Knowledge, Attitude and Practices (KAP) Of Digital Dentistry Among Dental Students. 100 BDS pursuing students were included in the study, out of which most of were interns.

Everyone in the dental field is looking forward to the future with great excitement because digital dentistry has brought about so many developments in recent years.

The necessity to assess the depth and breadth of dental applicants' knowledge and attitudes toward digital dentistry is growing as a result of the continuous technological advancements that affect the general

It should come as no surprise that 98% of participants anticipated using digital dentistry in the future. It is anticipated that this will forecast interest in digital technology, particularly from a digital perception of this generation. The remaining 2 percent who did not anticipate using digital technology might be a sign that they have a clear idea of the conditions of the practice they plan to join as a provider. It might also be the result of mistrust of technology or ignorance about digital dentistry.[8]

To find out if the students had up-to-date knowledge before entering the field, they were asked questions about how they see and use digital dentistry. According to the findings, the majority of students favor digital dentistry over traditional, substituting IOPAR with RVG. This demonstrates a readiness to acquire new technology and apply them as reliable

means of providing healthcare.[9]
Yet, 21% of students believe that digital dentistry can greatly enhance the quality of dental care. This could be a result of insufficient comprehension of tec hnology, overshadowed by the thrill of simply engaging with a new digital instrument.
A solid grasp oftechnologies enables effective decisio n-making for personalized dental care delivery instead of viewing technology merely a s an end goal. To safeguard dental education fromthe influence of themarketing sector, greater focus should be directed towards training dental educators and their teams on new technologies. Furthermore, emphasis should also be given to incorporating this kind of information into the curricula of dental classes and seminars.
In recent years, there has been a rise in both the ability to train dental students and the enthusiasm for pursuing dentistry among potential students. The students were inquired about their anticipations regarding the quantity of dental schools, encompassing digital dentistry.[10] In this research, the majority of students believed that the sources of information about the progress in digital dentistry are journals, conferences, and webinars. This suggests that the dentistry curriculum is not an adequate element in shaping students' understanding of digital dentistry. [11] It might also suggest a distrust in institutional programs to adapt to advancing technology.
The aim of this research was to tackle the deficiency in knowledge and awareness regarding digital dentistry among dental students. It offers crucial insights for dental educators to anticipate future developments in dental curricula and practices, enhancing teaching methods and the admissions procedure.[12] Consider the constraints of this study, which are affected by demographics and personal interpretations linked to how each question is worded. A broader survey involving dental students and first-year dental students can provide valuable insights for comparing the knowledge acquired over a brief period and may identify areas where misconceptions still need to be corrected.

CONCLUSION

The results of this study show a favourable outlook on digital dentistry and a willingness to explore new technologies in dentistry. This interest can foster an enriching learning environment, support innovative teaching strategies, and welcome upcoming dental academicians. Nonetheless, it is essential for the dental curriculum to emphasize the significance of digital dentistry by incorporating critical thinking, self-directed learning, and comprehension of research.

DECLARATIONS

Ethical statement

This study was performed in line with the principles of the Declaration of Helsinki.

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Declaration of competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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RESEARCH ARTICLE

KNOWLEDGE, ATTITUDE, PRACTICE AMONG PAAN VENDORS IN KARAD REGION REGARDING HARMFUL EFFECTS, LAWS AND REGULATION REGARDING TOBACCO PRODUCT - "A QUESTIONNAIRE STUDY"

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ABSTRACT

Background: Tobacco use is a widespread public health issue in India, significantly contributing to preventable deaths, poverty, and a heavy healthcare burden on families and the nation. Despite existing legislation like the Cigarettes and Other Tobacco Products Act (COTPA) 2003, tobacco products remain easily accessible, especially through local paan vendors. This availability, coupled with a lack of awareness and weak enforcement, perpetuates the problem, especially among youth and in rural regions.

Objective:

To assess the Knowledge, Attitude, and Practices (KAP) of paan vendors in the Karad region regarding the harmful effects of tobacco and the laws regulating its sale and distribution.

Methodology:A cross-sectional, questionnaire-based study was conducted among 100 paan vendors from both urban and rural areas of Karad using convenience sampling. The questionnaire, comprising 15 pre-tested questions translated into Marathi, assessed demographic data, knowledge, attitudes, and practices related to tobacco and relevant laws. Data were collected via personal interviews and analyzed using SPSS version 23 with descriptive statistics.

Results: All participants were aware of the harmful effects of tobacco, yet 75% consumed tobacco themselves. Only 64% knew the legal age for selling tobacco products, and a mere 32% asked minors for age proof. About 31% were unaware that tobacco sales are prohibited within 100 yards of educational institutions. Although 81% knew about legal punishments, most vendors lacked proper licenses and reported no regulatory inspections. Peer pressure and stress were reported as major reasons for tobacco consumption.

Conclusion: While awareness of tobacco's health risks is high among paan vendors, adherence to COTPA regulations is lacking due to poor enforcement and negligence by authorities. Most vendors do not follow legal protocols, sell to minors, and continue malpractices such as selling loose tobacco. There is an urgent need for stricter surveillance, enforcement of laws, and community-based awareness programs. Active involvement of all stakeholders, especially government agencies and educational institutions, is essential to mitigate this public health challenge and reduce tobacco-related morbidity and mortality in India.

Keywords*:* Paan Vendors, laws and regulation, TOBACCO

INTRODUCTION

Tobacco is the common name for species of plant in the genus *Nicotiana* of the family *Solanaceae*, and the general term for any product prepared from the cured leaves of these plants. More than 70 species of tobacco are known, but the chief commercial crop is *N. tabacum*. Tobacco has long been used in the Americas, with some cultivation sites in Mexico dating back to 1400–1000 BC.[1] Tobacco was introduced in India by the Portuguese 400 years ago. Since then tobacco consumption has continued to rise in India. Different types of tobacco products are available in market.

Tobacco contains various chemical compounds for example O-containing compounds (75.70%), followed by the N-containing compounds (12.98%), miscellaneous compounds (10.61%, including the category "Others" at 1.21%), and finally the hydrocarbons (0.71%).[2]

India's Tobacco Board is headquartered in Guntur in the state of Andhra Pradesh.[3] India has 96,865 registered farmers who cultivate tobacco crops[4] and many more who are not registered but illegal cultivate it. In 2010, 3,120 tobacco product manufacturing services were functioning in India. 0.25% of India's cultivated land is used for tobacco production.[5] Since 1947, the Indian government has covertly supported growth in the tobacco industry. India has seven tobacco research centres, situated in TamilNadu, AndhraPradesh, Punjab, Bihar, Mysore, and West Bengal.

The extensiveness of tobacco use is a major public health menace leading to premature deaths, poverty, and healthcare burden to the family and the nation. Tobacco use contributes to poverty by diverting household spending from basic needs such as food and shelter to tobacco. In some countries children from poor households are employed in tobacco farming to boost family income. Various forms of tobacco are used all over the country both in chewable and non-chewable form that is smoking e.g bidi, cigarette, chillum, chutta, dumthi, gudaku, hookah, hookli, kaini, mainpuri tobacco, paan, snuff, zarda. In India, the tobacco industry can be divided into three distinct and profitable sectors: a. bidis, b. smokeless tobacco, c. cigarettes out of which in India the use of smokeless tobacco (ST) is nearly three times as common as smoking. India is known for its diversity of tobacco products. Smokeless tobacco in India remains by far the most prevalent form used (38%) followed by bidis (48%) and cigarettes (14%).[6] Tobacco-related health issues are avertible but still tobacco is the leading cause of mortality for more than 7 million people per year.[7] According to the report of World Health Report, 2002 by WHO, developing countries already account for half of all deaths attributable to tobacco. This proportion will increase to 7 out of 10 by 2025 because prevalence of smoking has been rapidly increasing in many developing and underdeveloped countries even though it is decreasing in high-income countries. If the pattern of smoking all over the globe doesn't change, more than 8 million people a year will die from diseases related to tobacco use by 2030.[8]

Tobacco has deleterious effects on general health as well as on oral health, mostly on the oral mucosa and the periodontal attachment structures of the teeth. The most common oral problems affecting people who consume tobacco are development of white patch on the soft tissue in the mouth (called smoker's keratosis), poor healing after tooth removal (known as dry socket), tooth loss, poor healing after mouth and gingival surgery, decreased mouth opening, staining of teeth, halitosis, etc. if it continues, leads to precancerous lesions and condition ultimately resulting into various cancers of the oral cavity which today is one of the top three cancers in India, number one among all cancers in men and third highest among female cancers. The single largest cause of preventable death is nothing but the tobacco. These disease are very common because tobacco and its products are easily available in small road side shops[9] and for all age groups despite having strict laws and regulation implemented by government of India i.e Cigarettes and other Tobacco Products Act 2003 (COTPA 2003) (Prohibition of Advertisement and Regulation of Trade and Commerce, Production, Supply and Distribution), to reduce tobacco use.

Hence the present study was carried out to assess the Knowledge, Attitude and Practices (KAP) among Paan Vendors in Karad region regarding the harmful effects and laws & regulations regarding the use and sale tobacco products.

METHODOLOGY

This study was a cross-sectional questionnaire study. Data was collected in October 2024. The target population for the study comprised 100 paan vendors, who were selected by convenience sampling technique in Karad city.

The questionnaire comprised two sections, where the first included 4 questions related to demographic data. The second section concentrated on 6 questions to assess knowledge, 4 questions to assess attitude, and 5 questions to assess practice regarding the same. Demographic data includes the name and age of the paan vendor, gender, and qualification. Out of 15 questions, 8 questions were binomial i.e. having two options: yes or no type.

In order to attain the highest possible accuracy, participation in this study was voluntary and the questionnaire was anonymous and self-administered because some study subjects refuse to give their demographic detail in the fear of getting arrested or fear of their shop being raided. The participants were informed the importance of answering the questions correctly and were assured of their confidentiality. Data was collected by personal interview.

The questionnaire was written in English and then translated to the local Marathi language and again retranslated into English to test the linguistic validity. Most of the study participants were illiterate or partially literate. For such subjects, one assistant was employed to explain the questionnaire in the regional language.

Approval was obtained from the Institutional

Ethics Committee prior to onset of the study and after designing the protocol and questionnaire (058/2024-2025). The respondents were informed about the purpose of the study using information sheets written in English and Marathi. In each case, signing or thumb print the consent form was a prerequisite that affirmed their participation in the study based on informed consent. The respondents were also informed that their participation was voluntary, and they could stop their participation at any stage in case they felt any question was infringing on their privacy. The responses were anonymized to keep all the information confidential.

The descriptive statistics were applied in characterizing the respondents. The data was entered into the Statistical Package for Social Sciences (SPSS) program version 23 by IBM, Armonk, New York, United States for descriptive analysis. The primary methods included frequencies and percentages. Presentation of the results was in tables, charts, and graphs to ease interpretation and facilitate drawing of conclusions.

RESULTS

Demographic finding

Table 1 illustrates the demographic characteristics of the participants. The study aimed to determine the age distribution of the respondents based on four quantifications: 20-30 years, 30–40 years, 40-50 years, 50-60 years, 60-70 years.

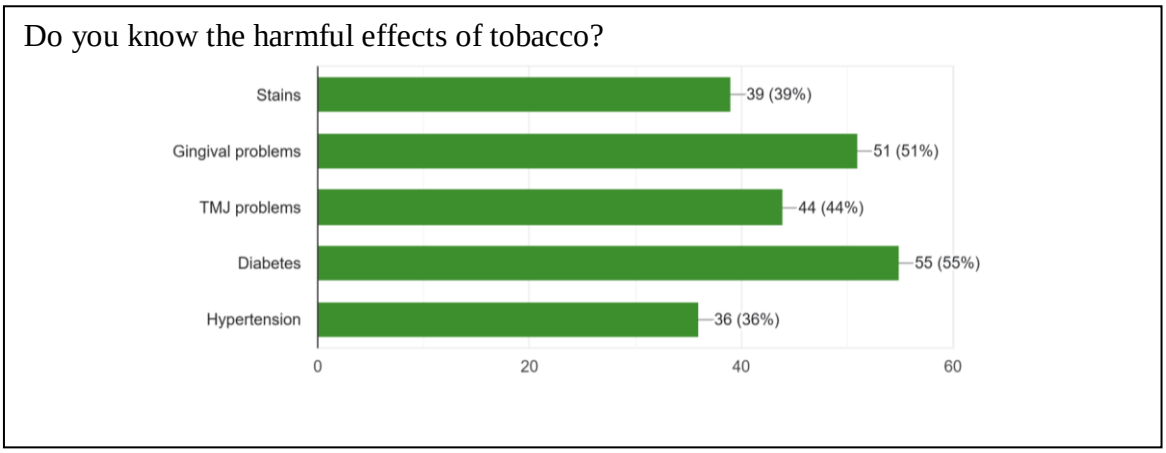
The findings indicate that 40% of participants were aged between 30-40 years and 38% of participants were aged between 40-50 years. Males comprised most of the respondents, 94% while females represented only 6%.

According to Graph no. 1, all the participants were aware about harmful effects of tobacco still 75% were consume tobacco product. Graph no. 2, according to 63% participants habit followed by peer pressure, show-off was the main reason to buy tobacco product. Graph no. 3 showed that 64% of participants were know about legal age to which they can sell tobacco products i.e. 18 years According to participants. Graph no. 4 revealed that 31% participants were not aware about the law about at what distance tobacco shop can be open from educational institutes and hospitals i.e 100 yards. Graph no. 5, the 30% participants don't know the fine being imposed on people smoking in public places. According to 37% of participants, the sale of Tobacco products hampered due to increased taxations by the government. Results also showed that 64% of the paan vendors who were participated in the study were aware about age of selling tobacco still only 32% were asked for age proof to minors. All the participant were aware about ban on tobacco products, but only 81% were known punishments regarding it.

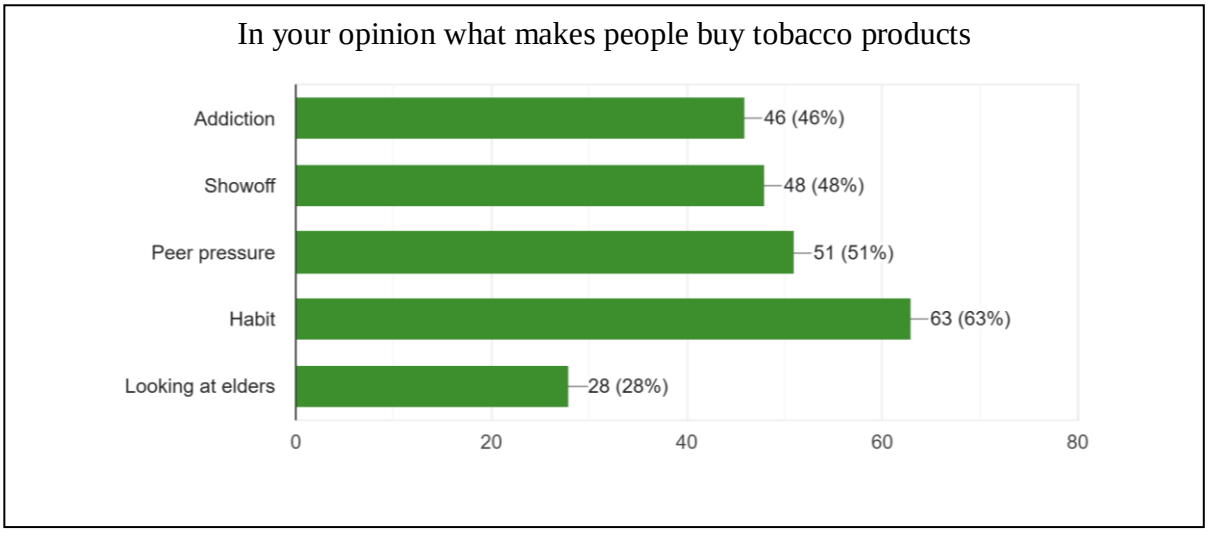
Table 1. Demographic Data of the Respondents

Demographic Factors			Frequencies (f)		Percentages (%)
Age	20-30 years		10		10
	30–40 years		40		40
	40-50 years		38		38
	56-60 years		11		11
	60-70 years		1		1
Gender	Female	Graph	6	No. 1	6
	Male		94		94

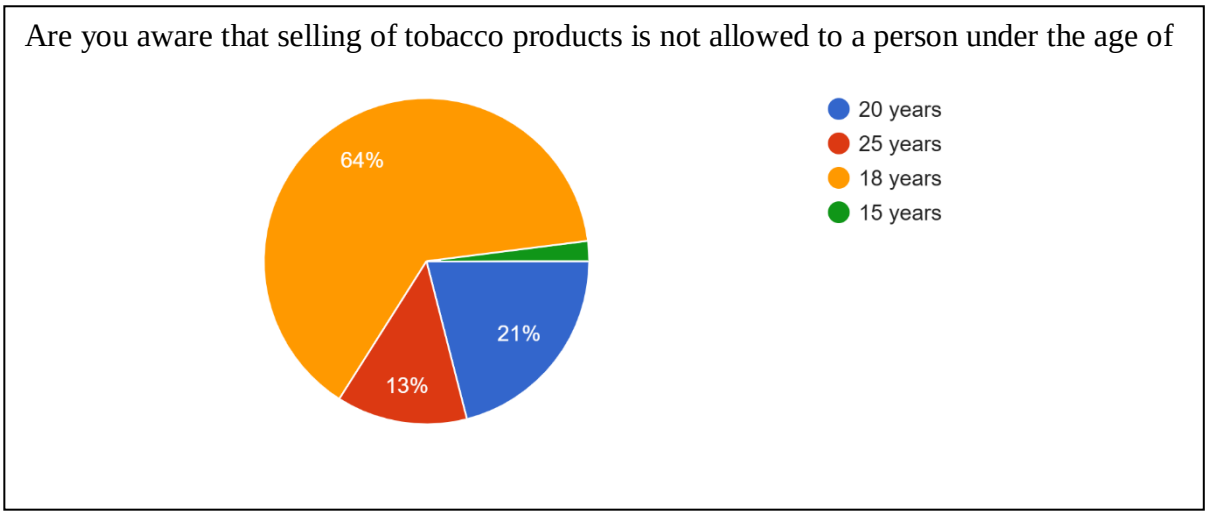
Graph N1



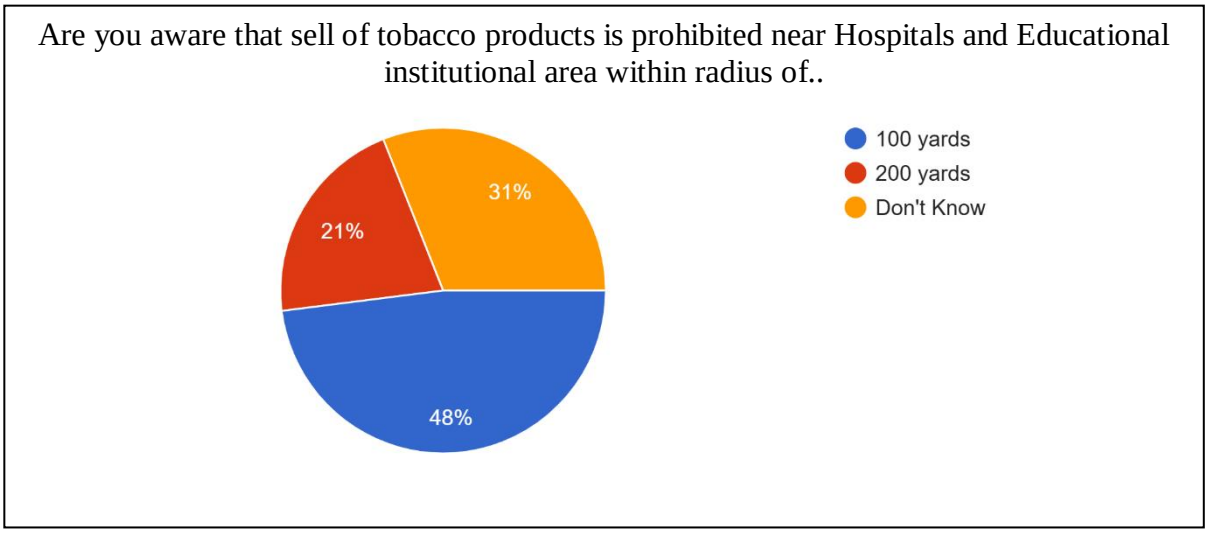
Graph N2



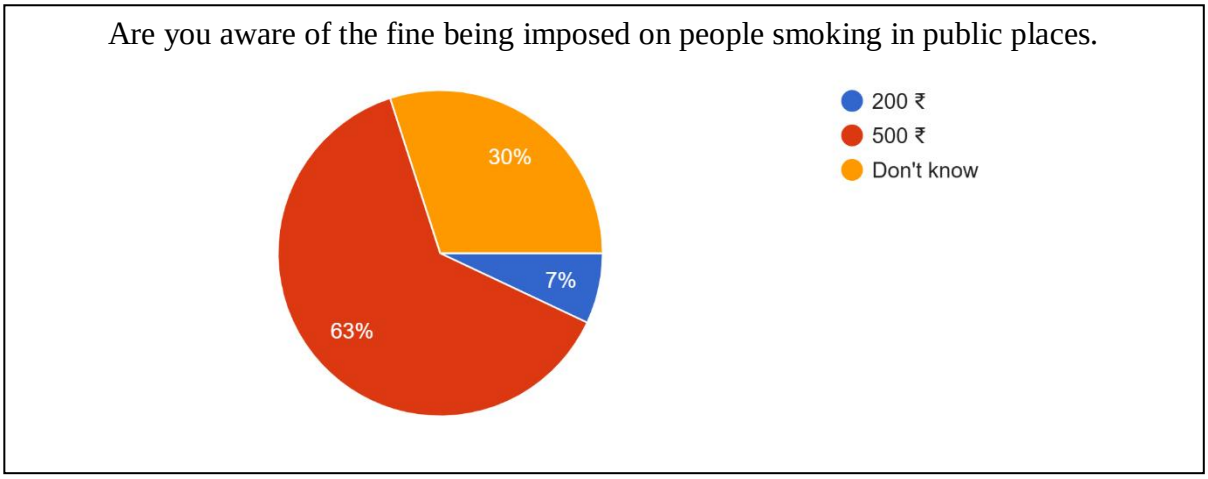
Graph N 3



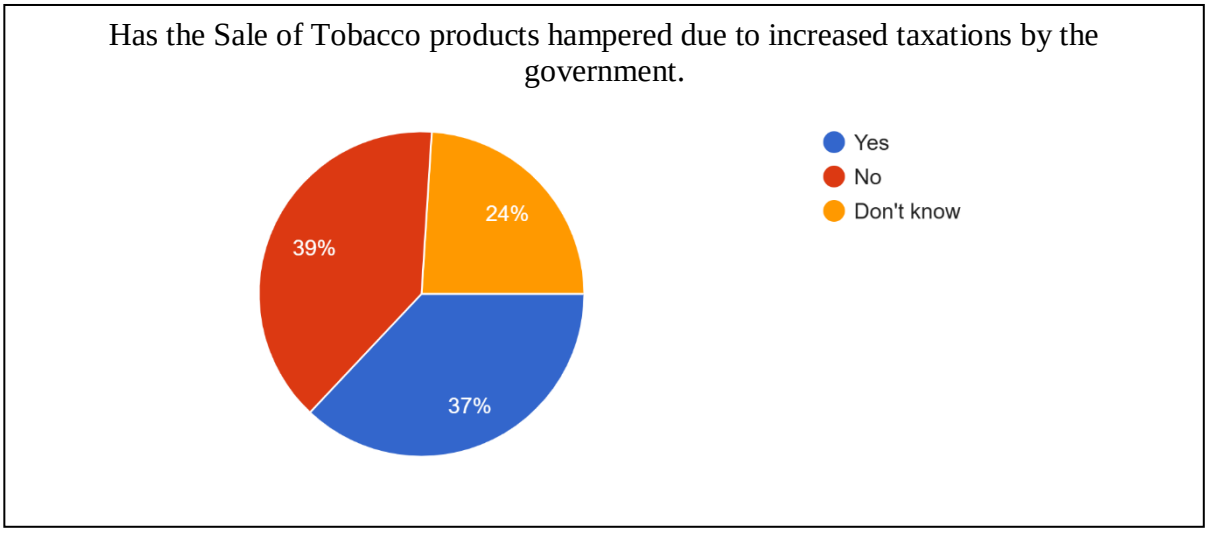
Graph N 4



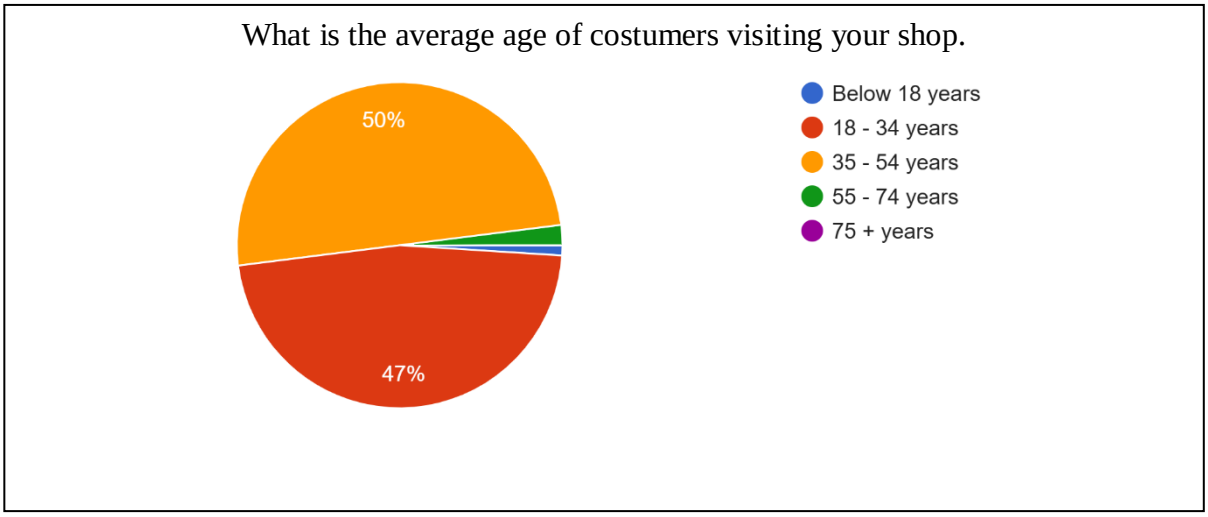
Graph N 5



Graph N 6



Graph N 7



DISCUSSION

As this was a cross sectional questionnaire study focuses on KAP related to harmful effects, laws and regulation regarding tobacco products in Karad region. 100 paan vendors were included in the study, out of which most of were partially literate. Hence there must be lack of education and awareness regarding the same.

Tobacco leads to several symptoms which cause precancerous lesion and condition which ends up in death of a person. This all happened because of availability of tobacco at minor age, long term use of tobacco, etc. according to this study, none of the participants ask for age proof to minor while selling tobacco, which was given by COTPA 2003. In this study all participant never asked for age proof to minors, which is in line with results of the study conducted by *Arnold H. Levinson, Joseph G.L. Lee, Leonard A. Jason, Joseph R. DiFranza.*[9] According to opinion of 58% participants, stress rendered people to by tobacco products which is similar to study conducted in medical students.[11]

COPTA 2003 prohibits the sale of tobacco products within 100 yards of the outer boundary of educational institutions and bans the advertising of tobacco products at most locations (except point of sale with specific guidelines), the results of this study indicate that 48% participants were aware about this act which is in similarity with the study conducted by *J.L. Elf a, B. Modi, F. Stillman, P. Dave, B. Apelberg* in Ahmedabad.[12]

In current study, there was 37% drop in sale of tobacco products due to increase in taxation as mentioned by respondents. This results are in harmony with some study conducted.[13,14] All participants had knowledge about harmful effects of tobacco.[15] About 47 % people visited to shop were of age between 18-34 years which is similar to the study result showing around 54% people consume tobacco products in age between 15-34 years.[16] The result of this study revealed that 31% participants had no knowledge about the location of shops selling tobacco products within a radius of 100 yards of the educational institutions is not permitted, as per Section 6b of COTPA, 2003.[17] All of the paan vendors reported that there was no visit or inspection of the shop by any regulatory agencies (State FDA or Municipal Corporation Authorities)which indicates an alarming level of negligence on the part of the government.

CONCLUSION

It can be concluded that, despite all the paan vendors knowing about harmful effects of tobacco products, 75% of paan vendors themselves consumed tobacco in one form or another 1Study also point to a grave lacunae in following of laws and regulation guidelines by the paan vendors in the sale of tobacco, which is evident from the facts that only 63% of the paan vendors poses some sort of licenses to sale and there was no visit by any authorities or government agency for inspection. This resulted in serious malpractices which is evident from the fact that none of the paan vendors ever asked for any age proof to minors and have also sold loose tobacco products in clear violation of guidelines. The attitude of paan vendors was also found to be unfavourable since none of them encouraged anyone to quite their habit. This shows that paan vendors are aware about some laws and regulations of tobacco sale but their attitude and practices point toward a repeated tendency toward these rules and regulation especially in the absences of any government authorities. Thus it can be concluded that there is an urgent need of stricter implementations of the laws by active surveillance & regular inspections by the Government Authorities.

It can also be concluded that despite stringent measures laid down by the existing laws, hardly any of it is being practised or enforced on the ground. There is adequate dissemination of the knowledge of the laws but there is little implementation of the same. There needs to be stricter enforcement by the Government agencies especially in peripheral areas of cities or rural areas. There is also an urgent need to conduct programs in schools & colleges to spread awareness among younger section of the society, regarding the menace of tobacco. However to ensure successful curbing of this menace, there must be active participation from every stakeholder affected by this ‘epidemic’ of tobacco addiction.

DECLARATIONS

Ethical statement

This study was performed in line with the principles of the Declaration of Helsinki.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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REVIEW ARTICLE

BIOMATERIALS FOR ORAL REHABILITATION: NARRATIVE REVIEW

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ABSTRACT

Background: Recent progress in dental materials science has resulted in the creation of aesthetically pleasing and durable materials, which enhance the quality and lifespan of prosthetic restorations. In conjunction with these advances, nanotechnology and bioactive coatings are being increasingly employed to enhance osseointegration, minimize microbial colonization, and tackle biomechanical challenges in prosthodontics and implantology. This thorough review aims to compile current research on ceramics, polymers, nanoparticles, and implant biomaterials, concentrating specifically on their mechanical, biological, and surface-modification characteristics.

Materials and Methods: The literature was reviewed to assess modern applications of ceramic (e.g., monolithic zirconia), polymeric (such as PMMA and PEEK), and nanomaterials in prosthodontics. Key areas of focus included mechanical performance under stress (analyzed via FEA), adhesive systems, biofilm resistance, cytocompatibility, wear and aging characteristics, as well as the clinical potential of innovative implant surface treatments. Studies on in vitro, in silico, and chemical surface modifications were evaluated, including finite element modeling, artificial aging, and nanoparticle-reinforcement methods.

Results: Monolithic ceramics exhibit superior translucency and aesthetic qualities, although stress concentration remains an issue at the margins of restorations. PMMA displays favorable processing properties but has limitations in toughness and biostability, with enhancement approaches involving the use of nanofillers and copolymer systems. PEEK has emerged as a viable metal alternative due to its elasticity and biocompatibility. The incorporation of nanoparticles significantly enhances antimicrobial efficacy and wear resistance. Modifications to the surfaces of titanium implants using nanostructured coatings and protein functionalization may improve the osteoblast response and osseointegration. The guided implant surgical protocol, coupled with the socket-shield technique, may show potential in preserving bone structure and enhancing implant longevity.

Conclusion: All materials employed for oral rehabilitation must exhibit satisfactory aesthetic, biomechanical, and biological traits. Innovations in ceramics, polymers, and nanotechnology are transforming the field of prosthodontics and implant dentistry.

***Keywords*:**Zirconia, PMMA, PEEK, dental biomaterials, dental implantology, bioactive coatings

INTRODUCTION

The ongoing progress in the science of dental materials has notably influenced prosthodontics and oral rehabilitation. However, the accurate evaluation of prosthodontic materials continues to be a challenge because of the subjectivity and variability associated with clinical assessment standards. This complexity is exacerbated by the intricate intraoral environment, where materials encounter mechanical stresses, temperature changes, moisture, and microbial activity. Recently, the combination of computational methods in research on dental biomaterials has emerged as a promising avenue to improve the precision and consistency of material performance evaluations [1-3].

A growing array of innovative biomaterials like titanium and its alloys, yttria-stabilized zirconia (YTZP), zirconium-reinforced lithium silicates, lithium-disilicate ceramics, and reinforced PMMA has been developed to meet clinical needs regarding aesthetics, durability, and biocompatibility. Additionally, new technologies that utilize nanoparticles, nanostructured coatings, and hybrid fillers have demonstrated potential to enhance mechanical strength, antimicrobial properties, and bioactivity. Nevertheless, even with these advancements, our comprehensive understanding of their long-term performance in the oral environment remains limited [4-6].

To fill these gaps in knowledge, laboratory studies and computational modeling, especially finite element analysis (FEA), have become increasingly popular. These methods enable the simulation of stress distribution, fatigue performance, and the interactions between biomaterials and biological tissues under conditions that mimic clinical situations. Furthermore, techniques like additive manufacturing and nanotechnology are paving the way for customized prosthetic designs that offer improved precision and functionality [2,7].

This study examines the current state of dental biomaterials, highlighting the importance of computational assessments in evaluating their characteristics and informing clinical choices. It aims to compile existing evidence while pinpointing critical areas for forthcoming research and advancements in prosthetic and implant dentistry.

Ceramics

Newly developed multi-color monolithic ceramics are highly durable, translucent, and aesthetic, which makes them suitable for applications where such properties are essential. As a result, the rising adoption of conservative treatment involving adhesively bonded monolithic restorations is warranted. Further studies need to be conducted and published, building on prior research concerning prosthetic restorations, particularly regarding implant-supported constructions|[1]. Beyond the properties of the material itself, the stress concentration is a significant aspect influenced by the nature of marginal preparation, endodontic therapy, and the type of superstructure utilized. With respect to stress levels, modern approaches like the biologically oriented preparation technique (BOPT) may offer a favorable option for anterior monolithic zirconium crowns; however, it is important to note that the highest stress levels occur at the edge of the restoration [2]. From a biological standpoint, recent cytomorphometric, bacteriological, and hygiene-related studies have indicated that zirconium restorations perform better than traditional Co-Cr-based ceramic restorations [3].

Research indicates that various erosive factors, including the low pH found in many beverages, may adversely affect ceramic surfaces. This issue should not be overlooked, and patients receiving prosthetic treatment should be informed about the potential corrosion effects. It has also been observed that prolonged storage can lead to increased surface roughness in ceramics [4].

Polymers

Relevant areas of research for examining the factors behind the decline in the retention potential of materials include thermal variation and water dispersion. Consequently, it was observed that retention loss was more pronounced in nylon and polyetheretherketone (PEEK) compared to polyvinyl siloxane (PVS). Nevertheless, all the polymeric materials studied showed a reduction in retention after one year of artificial aging. Therefore, further research on attachment systems suitable for the repair of abutments in at-risk patients is still warranted [5-7].

Employing an adhesive system facilitates the establishment of a robust bond between copings and resin cement; resin cement alone should not be utilized. An adhesive must be incorporated into clinical practice to achieve improved outcomes. Thus, investigations into tensile bond strength should be conducted using various materials that hold similar indications [8].

The properties of PMMA make it an ideal base material for prosthetics. This material is applicable in denture rebases, reliners, maxillofacial prosthetics, orthodontic devices, temporary restorations, and surgical splints. The advantages that render denture base materials popular include aesthetics, accurate fabrication, and a straightforward manufacturing process. Additionally, they should be easy to repair, readily available, and cost-effective. Despite its many beneficial traits, PMMA has certain drawbacks. A significant limitation of PMMA is its inadequate toughness, leading to a frequent need for repairs over the course of a year. Another common issue is that some people may experience allergic reactions to acrylic resin, which can be addressed by modifying the bases of resin prostheses [5-9]. Consequently, further research is necessary to evaluate the biocompatibility and toxicity of PMMA-based dental materials.

The physical properties of PMMA, including its durability, strength, lightweight nature, and high impact toughness, surpass those of glass and polystyrene, while its environmental resistance is significantly better than that of other plastics intended for biomedical use [5,7,10]. However, dental polymeric materials are susceptible to hydrolysis, which can lead to soaking and degradation, which is a notable disadvantage [7,10,11] (Figure 1).

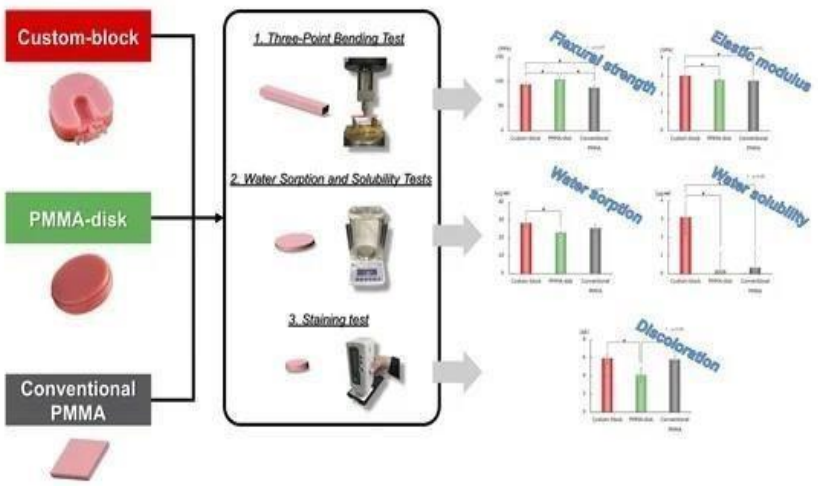


Figure 1. Bending, water sorption, and staining of custom-block, PMMA-disk and conventional PMMA [11].

Although traditional PMMA and resin composites have been successful, high-density polymers are gaining traction in dentistry. PEEK (polyether ether ketone), a member of the polyaryletherketone (PAEK) family, is a polymer biomaterial utilized as a substitute for metals and less durable polymers in both fixed and removable prosthetic applications. The elasticity modulus of PEEK is lower (approximately 4 GPa) compared to that of ceramics and metals (around 60–200 GPa), which offers a better alternative for absorbing functional chewing forces. Furthermore, PEEK can replace standard superstructures and denture base resin materials with advanced mechanical properties and reduced discoloration. Additionally, composites and titanium dioxide may be incorporated to enhance its aesthetic qualities. PEEK also exhibits excellent chemical resistance, allowing it to withstand high temperatures without significant degradation. Due to its very low allergenic potential, it infrequently triggers immune responses following intraoral use [8,9,12-15]. Thus, further investigations into this property could enrich the scientific literature and accurately showcase the advantages and drawbacks of its application in prosthetic and implant dentistry.

The use of various polymeric biomaterials in prosthodontics and dental implantology has grown due to their satisfactory mechanical and biological properties, which help in handling chewing loads; however, there are still several areas that need improvement for dependable clinical use [16-20]. A rapidly advancing topic is the additive manufacturing technique, which enables dentists to produce intricate restorations and structures with high accuracy based on a digitally designed model. Different 3D printers and polymers are available for various applications [5,9,10] (Figure 2) [21]. Hence, the scientific literature could benefit from new insights regarding these materials in terms of processing, sterilization, dimensional stability, and the appropriate disposal methods for waste byproducts.

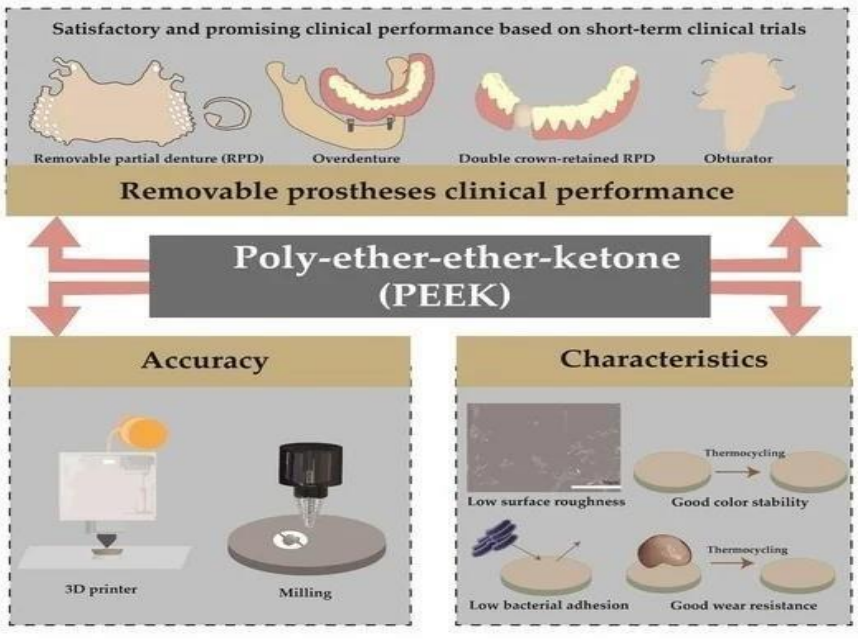


Figure 2. Fabrication of various prosthetic constructions from polymeric biomaterials [21]

Nanoparticles and nanotechnology

To lessen the colonization of bacteria and fungi on PMMA, a variety of active nanoparticles can be integrated into its composition before the final polymerization stage. Nanoparticles such as zirconium dioxide, silver, and platinum are currently being utilized within PMMA [10]. Silver nanoparticles can enhance the thermal conductivity of biomaterials, thus providing improved comfort for patients. Besides enhancing mechanical properties, the incorporation of ZrO_2 nanoparticles reduces biofilm adherence to denture bases and removable dentures. Depending on the concentration, the bioactivity of C. albicans biofilm can be significantly diminished, demonstrating anti-adhesion properties when a high concentration (5%) of Ag-NPs is employed [5,8,12,22] (Figure 3).

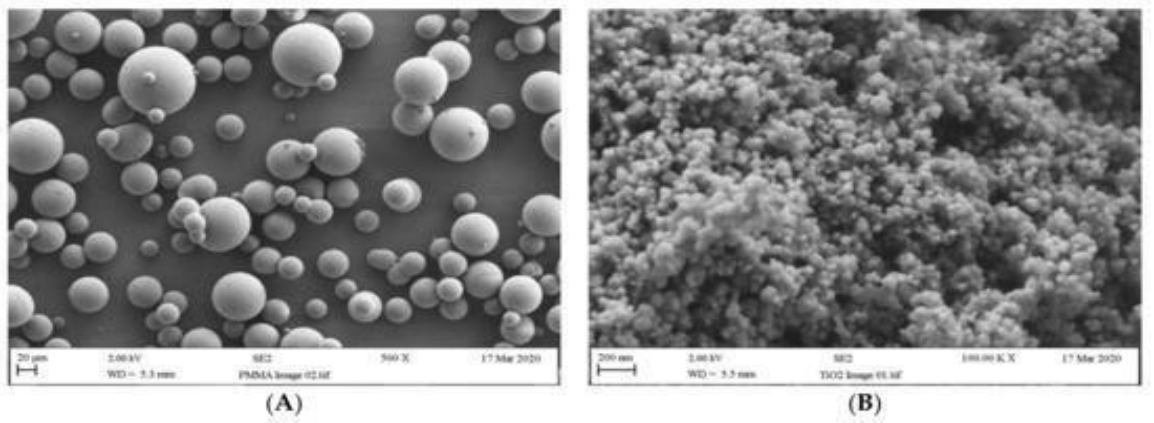


Figure 3. Particle/fibre size of (A) PMMA powder, (B) ZrO_2 nanoparticles, (C) TiO_2 nanoparticles and (D) E-glass fibre [22].

Apart from utilizing nanoparticles, biofilm formation can also be prevented by creating various polymeric nanofilms to coat biomedical devices. For instance, phospholipid polymers can help hinder the adhesion of S. mutans to denture bases 13-[15]. Results from in vitro studies indicate that nanocomposites can effectively eliminate both Gram-positive and Gram-negative bacteria without causing significant cytotoxic effects on osteoblasts, fibroblast-like cells, or adipose tissue-derived stem cells [12,17].

The application of nanoparticles as enhancements in traditional glass ionomer cement is another area that deserves consideration. Prior research indicated that incorporating 8% titanium reinforcement in glass ionomer cement reduces the wear rate by nearly 30%. However, there was no improvement in surface hardness. Factors such as the environment, mixing duration, and resin ratio may influence the mechanical properties of materials [23]. Exploring these factors could enrich the scientific literature and advance the field of dentistry.

When it comes to oral implant materials, titanium alloy has a slight disadvantage: it has relatively low wear resistance. Currently, nanostructured ceramic coatings are utilized to address this issue. A bilayered coating has shown a 200- and 500-fold improvement in wear resistance compared to the monolayer Al_2O_3-13TiO_2 and ZrO_2, respectively, due to its superior adhesion and reduced porosity [1]. The development and application of new coating layers in clinical settings should be pursued.

Researchers have employed various techniques to produce nano-crystallization on metal surfaces to enhance their biological activity. A nano-textured titanium surface was created using chemical etching techniques. Previous studies have explored the impact of nanotextured titanium surfaces on the proliferation, adherence, differentiation, and mineralization of murine preosteoblastic cells. Consequently, nanophase metals appear to hold significant potential in both prosthetic applications and implants, yet further investigation is needed. Research has found that nacre powder, derived from the inner layer of mollusk shells, fosters peri-implant osteogenesis in the tibiae of domestic pigs. Analyses using micro-CT and histological methods have demonstrated that this bioactive substance promotes effective bone formation around an implant surface. The incorporation of nacre powder, alongside surgical implant placement, could serve as an alternative strategy to enhance osseointegration[24-26]. Further in vitro studies will be valuable for examining various biomaterials for surface modification in dental implants.

Implant biomaterials and bioactive coatings

For implant osseointegration to be successful, suitable conditions must be established. A lack of sufficient blood supply at the implant/bone interface, caused by inadequate oxygen levels, can lead to the accumulation of electrons that may heighten the risk of infection and inflammation, ultimately resulting in the rejection of the implant. To improve the surface conditions for osseointegration and address challenges related to the contact with dental implant surfaces, low-temperature curing can be employed with high-resistance fiber-reinforced fillers and complex additives, or antimicrobial combinations involving thermoset polymers [20,28] (Figure 4).

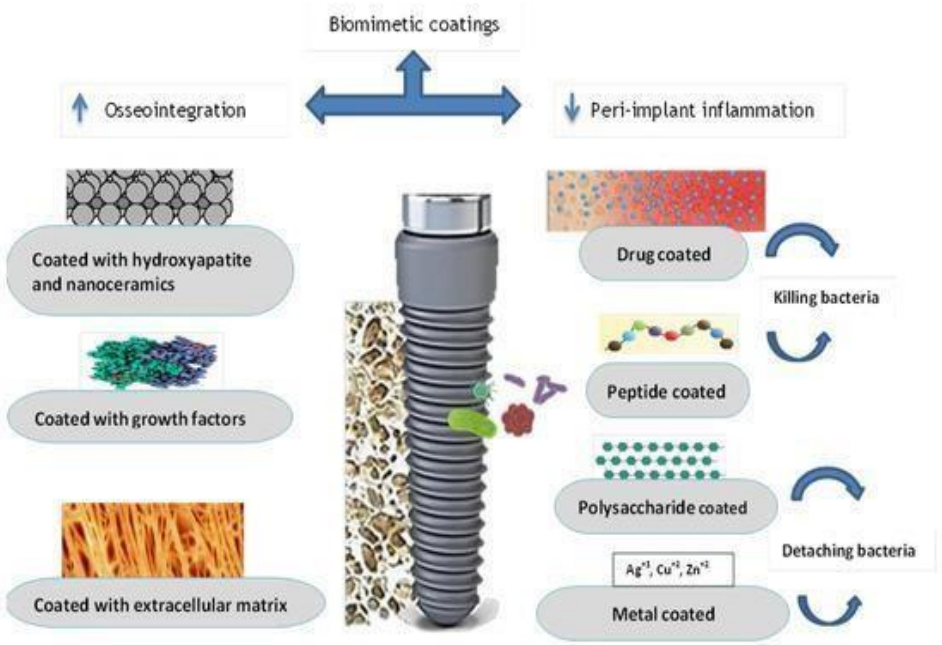


Figure 4. Implant surface biomimetic coatings for better osseointegration [28].

Researchers are currently focused on creating bioactive coatings for dental implants to enhance osseointegration by facilitating interactions between proteins, cells, tissues, and the implant surface. The use of appropriately coated materials, along with locally applied bone-resorbing medications in the tissues surrounding implants, significantly contributes to the success of dental implant procedures [20, 29]. It has been noted that these bioactive coatings can improve initial cell adhesion. Furthermore, nanofiber-based polymer coatings that contain antibiotics may also be used on implants to reduce the likelihood of implant failure, particularly in patients suffering from chronic periodontitis. At the same time, long-term release of antimicrobial medications such as metronidazole, ciprofloxacin, or minocycline has been facilitated by polymeric coatings [9]. However, certain mechanical properties of polymer coatings may be inadequate, and their effectiveness can be limited due to plastic deformation under high-stress conditions[8]. Therefore, additional research in this area is necessary. Dentin matrix protein promotes adhesion, cell growth, and encourages the differentiation of human stem cells along with the formation of a mineralized matrix. Hence, it is advisable to apply a biologically modified titanium surface with dentin matrix protein to improve osseointegration in titanium implants [26,30] (Figures 5,6).

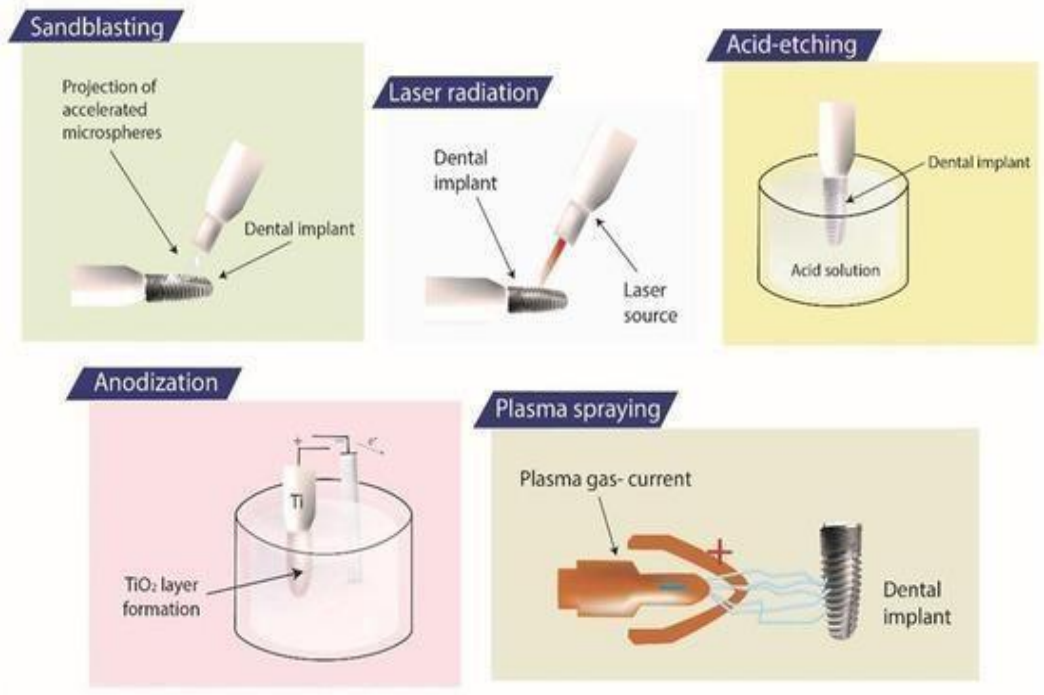


Figure 5. Dental implants' surface modification methods[30].

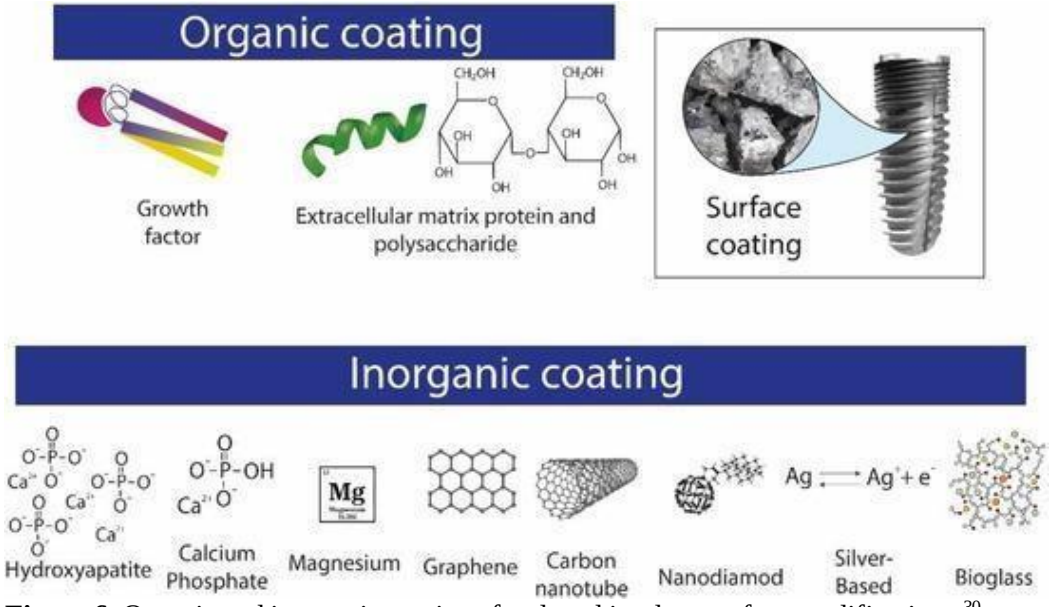


Figure 6. Organic and inorganic coatings for dental implant surface modifications [30].

As indicated in earlier studies, directly placing dental implants with bone graft materials did not halt peri-implant bone remodeling, and a decrease in bone thickness was still evident. Notably, the changes in the buccal alveolar ridge were more pronounced. Therefore, this technique can serve as an alternative to existing treatment protocols for implant placement in posterior regions [25]. In 2010, Hurzeler et al. presented an innovative approach known as the socket-shield technique, which aimed to prevent the diminishing of the facial ridge that frequently occurs following tooth extraction and immediate implant placement. This method is distinguished by its capacity to conserve a segment of the periodontal ligament next to the root fragment, thereby reducing the aesthetic impact of bone remodeling. Research has demonstrated that socket shielding is an effective means of preventing bone loss, subsequently eliminating the need for soft-tissue grafts. According to the referenced literature, socket shields should ideally measure at least 1.5 mm in thickness and preferably 2 mm. However, there is a scarcity of research investigating the impact of varying thicknesses of the root fragment when employing this technique. Thus, the stress levels on different root thicknesses combined with the socket shield method and rapid dental implant insertion were assessed utilizing finite element analysis (FEA) [31].

Our findings indicate that the guided implant surgical protocol offers advantages as the final results can be derived from a dependable treatment plan, minimizing marginal bone resorption and enhancing implant durability. The guided implant surgical (GIS) protocol plays a crucial role in avoiding flap elevation and ensuring optimal implant placement while safeguarding the marginal bone surrounding the implant. Subsequently, bioactive glass can be utilized in grafting to facilitate optimal bone formation as a potential scaffold biomaterial. Additional research into GIS will validate these findings and lead to tailored, refined treatment protocols for patients [32].

Regarding the mechanical response of bone tissue, achieving a uniform strain is essential and can be influenced by the various biomaterials utilized for reconstructing the edentulous maxilla. The use of zirconium, CoCr, and titanium contributes to a reduction in stress on zygomatic implants and prosthetic screws since these materials possess greater strength and superior mechanical properties compared to polymeric superstructures [33,34]. It is clear that advancements in prosthodontic technology heavily rely on progress in materials science. Indeed, the most crucial impact on fundamental scientific advancements and technological developments currently occurring in clinical prosthodontics is from nanomaterials. Through nanotechnology, materials can be miniaturized to the nanoscale, thereby improving various properties such as surface hardness, elasticity modulus, polymerization shrinkage, and filler content to enhance the functionality of established materials.

DISCUSSION

Currently, zirconium stands out as the most commonly used restorative material, resembling the color of natural teeth, while PEEK can serve as an alternative for more flexible superstructures. Zirconium crowns have been shown to endure three

times the wear from antagonists and exhibit superior color stability. These crowns demonstrate minimal displacement compared to those made from PEEK. However, PEEK prosthetic crowns exhibit minimal wear, better stress distribution due to plastic deformation, and enhanced color stability, suggesting that they could serve as a viable alternative to zirconium in crown production. The selection of material for denture fabrication is influenced by its intended use, thereby taking into consideration its characteristics, benefits, and drawbacks [9].

Researchers assessed how different implant biomaterials affect stress distribution in the bone surrounding implants. Upon comparing titanium and zirconia implants, they discovered that both the material of the implant and the implant system can affect how the superficial bone around the implant deforms when masticatory forces are applied. Nonetheless, this investigation did not explore the patterns of stress distribution in the peri-implant bone tissue related to the type of prosthetic material or various implant characteristics, such as diameter and thread design. Additionally, they did not examine the stress distribution patterns in the tissues supported by the implant concerning a particular surgical method [35].

Another study examined the physical characteristics of custom polymeric blocks created with a self-polymerizing resin (Custom-block), a commercially available CAD/CAM PMMA disk (PMMA-disk), and a heat-polymerizing resin (Conventional PMMA), which were assessed through three distinct tests. The findings revealed notable variations in flexural strength among the different materials. Notably, both the flexural modulus and water solubility were significantly elevated in the Custom-block. Additionally, the Custom-block exhibited a significantly higher water sorption compared to the PMMA-disk, although it showed no substantial difference from the Conventional PMMA. While this study focused on the flexural strength and solubility of various polymeric materials, it did not address the biocompatibility, toxicity, and degradation of various polymeric biomaterials in an oral cavity-like setting [11].

Researchers explored HA-PMMA composites through molecular docking as a biomaterial candidate for a dental implant in silico. The study concluded that hydroxyapatite-polymethylmethacrylate binding may increase osteonectin activity, as proven in silico. However, the study highlighted that further analysis must be carried out to investigate and clarify the given statements. Moreover, this study did not comprehensively evaluate the biocompatibility and toxicity of poly (methyl methacrylate) and its monomeric unit, methyl methacrylate, a crucial component in dental materials for interim prosthetic restorations [36].

The mechanical and aesthetic characteristics of three widely utilized dental implant materials: titanium, zirconia, and ceramic, were thoroughly evaluated. The assessment of mechanical properties such as tensile strength, elastic modulus, and fatigue resistance was conducted with advanced testing equipment. Additionally, fracture characteristics and biocompatibility were analyzed. However, traditional methods of examining dental implant materials have shortcomings in offering a complete understanding of material performance, particularly regarding complex atomic and electronic interactions [37].

CONCLUSION

All materials employed for oral rehabilitation must exhibit satisfactory aesthetic, biomechanical, and biological traits. Innovations in ceramics, polymers, and nanotechnology are transforming the field of prosthodontics and implant dentistry. Although promising, these materials come with particular limitations—whether mechanical, biological, or surface-related—that necessitate further investigation. Attention should be directed towards in vitro and computational analyses to optimize their clinical use, especially with regard to stress distribution, long-term performance, and biological integration. Continued development of bioactive coatings, additive manufacturing methods, and surface-engineered implants could establish new benchmarks in prosthetic and implant-supported rehabilitation. Furthermore, choosing the right prosthetic biomaterial and prosthesis design can influence modifications in the surgical treatment strategy.

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Informed consent statement
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Competing interests
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RESEARCH ARTICLE

ANTBIOTIC RESISTANCE OF DENTAL ABSCESS INFECTION IN PAEDODONTIC PATIENTS: A PROSPECTIVE STUDY IN A TERTIARY CARE DENTAL HOSPITAL

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ABSTRACT

Background: Dental abscesses are a common infection in paedodontic patients and are often managed with antibiotics in conjunction with dental interventions. However, the increasing prevalence of antibiotic resistance presents a significant challenge in effective treatment, leading to prolonged infections, complications, and increased healthcare burden.

Objective: This study aimed to evaluate the patterns of antibiotic resistance in bacterial isolates from dental abscesses in paedodontic patients attending Mahe Institute of Dental Sciences & Hospital.

Methods: A prospective cross-sectional study was conducted over a period of 3 months. Paedodontic patients presenting with clinical signs of dental abscesses were included. Pus samples were aseptically collected and cultured for bacterial isolation. Identified isolates underwent antibiotic susceptibility testing using the Kirby-Bauer disk diffusion method following CLSI guidelines.

Results: In the 25 samples collected, revealed the presence of multiple microorganisms, with a total of 25 isolates identified. *Streptococcus oralis/mitis* was the most prevalent organism, accounting for 6 isolates (24% of the total). Other significant organisms included *Staphylococcus hominis*, *Klebsiella pnaeumoniae*, *Citrobacter koseri*, *Peribacillus simplex*, and *Enterobacter cloacae*, each contributing 3 isolates (12% each). Additionally, *Enterobacter aerogens* and *Bacillus altitudinis* were identified, each comprising 2 isolates (8% each).

Conclusion: The study highlights a concerning level of antibiotic resistance among pathogens causing dental abscesses in children. These findings underscore the necessity for culture-guided antibiotic therapy and the development of local antibiotic stewardship protocols to prevent the escalation of resistance and ensure optimal patient outcomes.

Keywords*:* Dental abscess, paedodontics, microbiota, antibiotic resistance

INTRODUCTION

Apical abscess is the most common form of dental abscess and is caused by infection of the root canal of the tooth. It is usually localized intraoral, but in some cases the apical abscess may spread and result in severe complications or even mortality. The reasons why dental root canal infections can become symptomatic and evolve to severe spreading and sometimes life- threatening abscesses remain elusive.[1] Studies using culture and advanced molecular microbiology methods for microbial identification in apical abscesses have demonstrated a multispecies community conspicuously dominated by anaerobic bacteria. Species/phylotypes commonly found in these infections belong to the genera *Fusobacterium, Pavimonas, Prevotella, Porphyromonas, Dialister, Streptococcus and Treponema.*[1]

Most commonly prescribed drugs in pediatric dentistry are "Antibiotics." Among Dentists, there is a trend toward overuse of antibiotics for non-indicated clinical conditions. This insufficient knowledge of the appropriate clinical indications for antibiotic prescriptions promotes the overuse of antibiotics and contributes to the emergence of antibiotic resistance among children. According to the various surveys done on the dental students, dentists and pediatric dentists on the antibiotic prescribing practices, overall, adherence to the professional clinical guidelines was low.[2]

The American Academy of Pediatric Dentistry (AAPD) recognizes the increasing prevalence of antibiotic-resistant microorganisms and potential for adverse drug reactions and interactions. These recommendations are intended to provide guidance in the proper and judicious use of antibiotic therapy in the treatment of oral conditions. The use of antibiotic prophylaxis for dental patients at risk for infection is addressed in a separate best practices document. Information regarding commonly prescribed antibiotics can be found in AAPD's useful medications for oral conditions.[3]

All of the above factors might contribute to the emergence of antibiotic resistance among children. Children as young as 4 years were found to harbor multidrug resistant bacteria in their oral cavities.[4,5] Further, patients who received frequent prescriptions of amoxicillin, a common antibiotic in dental prescriptions also exhibited a higher rate of amoxicillin-resistant oral *Streptococci*.[6,7] In addition to the problem of antibiotic resistance, there are other complications associated with antibiotic prescribing in paediatric population. A significant proportion of practitioners prescribe mostly sugar-containing formulations at frequencies inconsistent with manufacturers recommendations, and for prolonged periods of time, that may reach 10 days.[8] The risk of developing diabetes in children due to sugar-containing medications cannot be overlooked. Moreover, generally, there is risk of development of allergy and asthma in children treated with antibiotics.[9,10] Early-life exposure to antibiotics is also thought to change intestinal microbiota, with subsequent adverse long-term effects like obesity.[11] Other complications include superinfections with *Candida* species[12] and photosensitivity.[11,13] Children are also at risk of gastrointestinal disturbances, like diarrhoea, which is generally more frequent with three-times-daily than twice-daily regimens.[12]

There are many reports on the complications associated with antibiotics abuse during the treatment of paediatric patients, particularly those related to antimicrobial resistance. The dental profession is no exception; there is growing evidence that dental practitioners are misusing antibiotics in the treatment of their paediatric patients.

Considering the above facts, this study is planned to identify the common micro-organisms in dental abscess and to pinpoint the best suitable antibiotic for those infections.

METHODOLOGY

This prospective cross-sectional study was conducted for a period of 3 months at Mahe Institute of Dental Sciences, Mahe. The ethical clearance for the study was obtained from the Institutional Review Board before starting the study. Informed consent was obtained and documented in standard proforma from consented subjects after individual counselling and providing information on the purpose of study, method of sample collection and their right of refusal. Demographic information such as age, gender, and oral hygiene, dietary habits was gathered in the standard proforma before the collection of samples. The samples were collected from 25 subjects.

Inclusion criteria: All consented patients with dental abscess in the paedodontic department was included for this study, irrespective of gender and age. The control group consisted of age and gender matched subjects in good oral health.

Exclusion criteria: Children with genetic disorders and parents who were not willing to give the consent was excluded from the study.

Sample Collection: Oral swabs were collected by Hilton method of abscess drainage and control samples by using a sterile dry cotton swab. The sample was immediately sent to microbiology laboratory for analysis.

Procedure: The oral samples collected was immediately processed in microbiology laboratory and placed in Brain heart infusion (BHI) broth. The broth was incubated at 37° C for overnight aerobically and was inoculated into Blood agar, Chocolate agar and Mac Conkey agar, culture media plates and incubated at 37° C. The mixed culture growths on the primary culture plates were the sub cultured individually in blood agar and chocolate agar, and each type of colonies grown was subjected for rapid phenotypic identification by automated bacterial identification system (MALDI TOF–MS).

Antimicrobial susceptibility testing: Antibiotic sensitivity testing was carried out by Kirby-Bauer disc diffusion method. Standard suspensions of the isolates were made and turbidity was matched to 0.5 McFarland standards. The isolates were inoculated on Mueller-Hinton agar (MHA) plates to perform a lawn culture using sterile cotton swabs and was allowed to dry for 5 – 10minutes. Antibiotic discs were then placed on the agar surface with appropriate spacing. The plates were inverted and incubated at 37°C for 18 – 24 hours. After incubation, the diameter of the zone of inhibition was measured and interpreted according to the Clinical Laboratory Standards Institute (CLSI) 2011.

Statistical analysis:

Statistical analysis was done by using SPSS software 2.0 version.

RESULT

Dental abscess samples were obtained from 25 individuals belonging to age group 7-12 years. Among the 25 samples 15 were female and 10 were males. The analysis of pediatric dental abscess samples was done using automated bacterial identification system (MALDI TOF- MS) revealed the presence of multiple microorganisms, with a total of 25 isolates identified. *Streptococcus oralis/mitis* was the most prevalent organism, accounting for 6 isolates (24% of the total). Other significant organisms included *Staphylococcus hominis, Klebsiella pnaeumoniae, Citrobacter koseri, Peribacillus simplex*, and *Enterobacter cloacae*, each contributing 3 isolates (12% each). Additionally, *Enterobacter aerogens* and *Bacillus altitudinis* were identified, each comprising 2 isolates (8% each) (Figure 1). All clinical specimens in the present study exhibited bacterial growth; majority of the organisms were Gram-positive organism. The growth observed was predominantly of Gram-positive cocci such as *Streptococcus mitis/oralis* (24%) followed by *Staphylococcus hominis*(12%). These samples were investigated for their antibiotic resistance pattern.

Antibiotic sensitivity testing was carried out by Kirby-Bauer disc diffusion method. After incubation and culturing, the diameter zone of inhibition was measured and interpreted according to the clinical laboratory standards unit (CLSI) and required data with traffic light chart was prepared.

The most commonly tested antibiotics against all the studied bacterial pathogen were amoxicillin/ clavulanic acid, ciprofloxacin, tigecycline, gentamicin, and clindamycin and others (as listed in the Fig 1). Other classes of antibiotics were often tested for diverse infection – colistin, fosfomycin, amikacin and cefoperozone were tested against *Staphylococcus, Peribacillus simplex, Enterobacter and Citrobacter.*

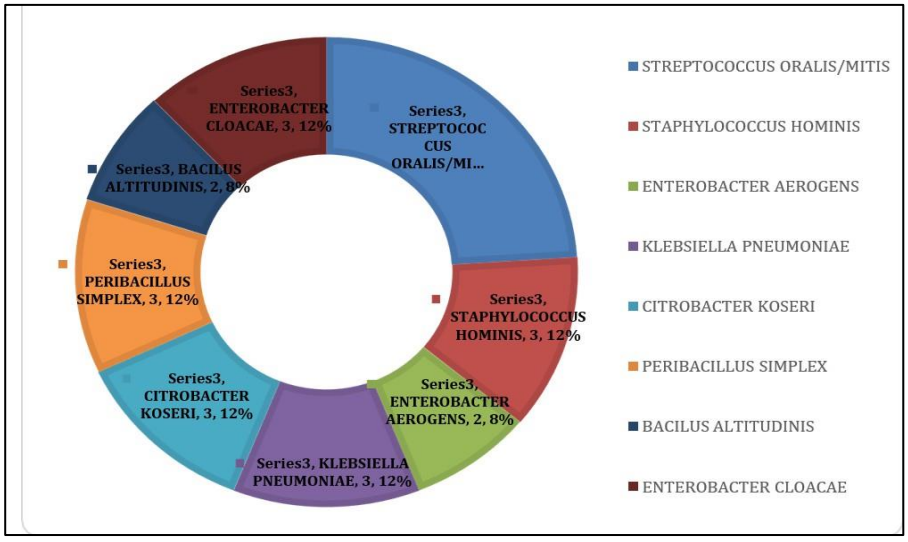


Figure 1. Identification and distribution of microorganisms isolated from pediatric dental abscesses.

Table 1. Antibiotic resistance pattern of Gram-positive microorganisms isolated from pediatric dental abscesses.				
	Gram positive microorganisms (n=14)			
	Streptococcus oralis/mitis(n=6)	*Staphylococcus hominis (n=3)*	*Bacillus altitudinis (n=2)*	*Peribacillus simplex (n=3)*
Amoxicillin/clavulanic acid	-	-	-	**R**
Piperacillin/tazobactam	-	-	-	**S**
Cefuroxime	-	-	-	**R**
Cefuroxime axetil	-	-	-	**R**
Ceftriaxone	**I**	-	-	**S**
Cefoperazone/sulbactam	-	-	-	**S**
Cefepime	-	-	-	**S**
Ertapenem	-	-	-	**S**
Imipenem	-	-	-	**S**
Meropenem	-	-	-	**S**
Amikacin	-	-	-	**S**
Gentamicin	-	**S**	**S**	**S**
Ciprofloxacin	-	**S**	**S**	**S**
Tigecycline	**S**	**S**	**S**	**S**
Fosfomycin	-	-	-	**R**
Colistin	-	-	-	**I**
Trimethoprim/sulfamethoxazole	-	**R**	**R**	**S**
Benzylpenicillin	-	**R**	**R**	-
Ampicillin	-	-	-	-
Cefotaxime	**S**	-	-	-
Levofloxacin	**R**	**S**	**S**	-
Moxifloxacin	**S**	-	-	-
Inducible clindamycin resistance	-	-	-	-
Erythromycin	-	**R**	**R**	-
Clindamycin	**S**	**S**	**S**	-
Linezolid	**S**	**S**	**S**	-
Teicoplanin	-	**S**	**S**	-
Vancomycin	-	**S**	**S**	-
Tetracycline	**R**	**S**	**S**	-
Chloramphenicol	**S**	-	-	-
Rifampicin	-	**S**	**S**	-
Cefoxitin screen	-	**R**	**R**	-
Ampicillin	-	**R**	**R**	-
Oxacillin	-	**R**	**R**	-
Cefazolin	-	**R**	**R**	-
Gentamicin high level	-	-	-	-
Daptomycin	-	**S**	**S**	-
Nitrofurantoin	-	**S**	**S**	-

Note: The chart uses a traffic light color scheme to indicate the resistance patterns of various organisms against selected antibiotics: red represents resistance (R), yellow indicates intermediate susceptibility (I), and green denotes susceptibility (S). This color-coded format provides a quick and clear visual representation of the antimicrobial effectiveness, highlighting critical areas of resistance and guiding therapeutic decisions.

Table 2. Antibiotic resistance pattern of Gram-negative microorganisms isolated from pediatric dental abscesses.

Antibiotics	Gram negative microorganisms(n=11)			
	Enterobacter aerogens (n=2)	*Klebsiella pnaeumoniae (n=3)*	*Citrobacter koseri (n=3)*	*Enterobacter cloacae (n=3)*
Amoxicillin/clavulanic acid	R	S	S	R
Piperacillin/tazobactam	S	S	S	S
Cefuroxime	R	S	S	R
Cefuroxime axetil	R	S	I	R
Ceftriaxone	S	S	S	S
Cefoperazone/sulbactam	S	S	S	S
Cefepime	S	S	S	S
Ertapenem	S	S	S	S
Imipenem	S	S	S	S
Meropenem	S	S	S	S
Amikacin	S	S	S	S
Gentamicin	S	S	S	S
Ciprofloxacin	S	S	S	S
Tigecycline	S	S	S	S
Fosfomycin	-	S	S	-
Colistin	I	I	I	I
Trimethoprim/sulfamethoxazole	S	S	S	S
Benzylpenicillin	-	-	-	-
Ampicillin	-	-	-	-
Cefotaxime	-	-	-	-
Levofloxacin	-	-	-	-
Moxifloxacin	-	-	-	-
Inducible clindamycin resistance	-	-	-	-
Erythromycin	-	-	-	-
Clindamycin	-	-	-	-
Linezolid	-	-	-	-
Teicoplanin	-	-	-	-
Vancomycin	-	-	-	-
Tetracycline	-	-	-	-
Rifampicin	-	-	-	-
Cefoxitin screen	-	-	-	-
Ampicillin	-	-	-	-
Oxacillin	-	-	-	-
Cefazolin	-	-	-	-
Gentamicin high level	-	-	-	-
Daptomycin	-	-	-	-
Nitrofurantoin	-	-	-	-

Note: The chart uses a traffic light color scheme to indicate the resistance patterns of various organisms against selected antibiotics: red represents resistance (R), yellow indicates intermediate susceptibility (I), and green denotes susceptibility (S). This color-coded format provides a quick and clear visual representation of the antimicrobial effectiveness, highlighting critical areas of resistance and guiding therapeutic decisions.

Predominant Gram-positive Organisms in dental abscess:

The Gram-positive isolates included *Streptococcus oralis/mitis* (n=6), *Staphylococcus hominis* (n=3), *Bacillus altitudinis* (n=2), and *Peribacillus simplex* (n=3). Resistance patterns among these organisms revealed *high resistance to amoxicillin/clavulanic acid*, cefuroxime, cefuroxime axetil, and fosfomycin. All isolates demonstrated sensitivity to tigecycline, ertapenem, imipenem, meropenem, linezolid, and chloramphenicol. Notably, *Streptococcus oralis/mitis* displayed resistance to tetracycline and levofloxacin, while *Staphylococcus hominis* showed resistance to erythromycin, cefoxitin, and oxacillin, indicating potential methicillin resistance.

Predominant Gram-negative Organisms in dental abscess:

The Gram-negative isolates included *Enterobacter aerogens*(n=2), *Klebsiella pnaeumoniae* (n=3), *Citrobacter koseri*(n=3), and *Enterobacter cloacae* (n=3). These organisms exhibited a high level of susceptibility to piperacillin/tazobactam, ceftriaxone, cefoperazone/sulbactam, ertapenem, imipenem, meropenem, amikacin, and ciprofloxacin. However, resistance to amoxicillin/clavulanic acid and cefuroxime was observed in *Enterobacter cloacae* and *Enterobacter aerogens*, suggesting variability in susceptibility among *Enterobacter* species. Intermediate susceptibility to colistin was observed across all Gram-negative isolates. Amoxicillin/clavulanic acid exhibited the highest resistance rates, with resistance observed in all tested Gram-positive isolates and a significant proportion of Gram-negative isolates, including *Enterobacter cloacae* and *Enterobacter aerogens*. Similarly, cefuroxime displayed high resistance across multiple organisms, particularly among Gram-positive isolates. The limited efficacy of these antibiotics suggests their reduced suitability for empirical therapy in pediatric dental abscess cases.

The high resistance rates to amoxicillin/clavulanic acid may reflect its frequent use in outpatient settings, leading to selection pressure and the emergence of resistant strains. These findings underscore the importance of performing antibiotic susceptibility testing to guide clinical decision-making and avoid overreliance on antibiotics with established resistance trends.

Antibiotic Susceptibility

Carbapenems (ertapenem, imipenem, and meropenem), tigecycline, and amikacin demonstrated the highest efficacy, with universal sensitivity among both Gram-positive and Gram-negative isolates. In contrast, amoxicillin/clavulanic acid and cefuroxime were the least effective, with widespread resistance observed in both groups.

DISCUSSION

Despite the increased incidence of odontogenic infections, there is a paucity of data on the bacteriological profile and the antimicrobial resistance pattern of the isolates. Such data are a prerequisite for the development of clinical recommendations and guidelines on the antibiotic prescribing practices to be adopted by dentists for the therapeutic management of odontogenic infections. In this study, Gram-positive bacteria dominated over Gram-negative bacteria, which is consistent with the previous report by Brescó Salinas et al in 2006.[14]

The most predominant organism isolated was similar to the study by Brescó-Salinas et al. in 2006, where Streptococcus species were the predominant organisms isolated.[14] Samples from the periapical region were collected, and when compared to abscesses in other regions (i.e., pericoronal and periodontal), the antibiotic resistance pattern was studied more conveniently from periapical abscesses. This is similar to the study conducted by Kuriyama et al in 2000, where periapical abscesses accounted for 78%, while the rest were 26%.[15]

Previous reports suggest that unnecessary antibiotic prescriptions significantly contribute to the development of antibiotic resistance. Furthermore, antibiotic prophylaxis is rarely addressed in dentistry.

Hence, periodic surveillance for antibiotic resistance, education on antibiotic stewardship, routine audits, and feedback could serve as essential interventions in hospital dental care and outpatient dental settings.

Brescó Salinas et al. in 2006 reported susceptibility rates of 91.4%, 34.2%, and 91.4% for *E. faecalis* against amoxicillin, azithromycin, and linezolid, respectively.[14] *Streptococcus oralis* showed 37% sensitivity to amoxicillin, according to Mahalle et al. in 2014.[16] While 76.95% were sensitive to azithromycin, according to Jagadish Chandra et al in 2017.[17] 60% sensitivity for linezolid was observed, according to Jindal et al in 2019.[18] For Enterobacter species, Abdulla et al. in 2009 reported 0%sensitivity to amoxicillin, which was also observed by Prakash et al in 2016 for amoxicillin-clavulanate.[19,20] These findings align with our study results.

Amoxicillin and amoxicillin–clavulanic acid are the empirical drugs commonly used for facultative anaerobes and aerobes. Tigecycline showed the highest

susceptibility rate of 100% in our study, followed by a 75% susceptibility rate for gentamicin, ciprofloxacin, linezolid, and teicoplanin in Gram-positive bacteria. Erythromycin or azithromycin are commonly used when patients are allergic to penicillin group antibiotics[21] Linezolid, an oxazolidinone and a recent reserve drug, is used against multidrug-resistant Gram-positive organisms.[21] Hence, linezolid was also tested alongside the commonly prescribed drugs. Linezolid is not recommended for Gram-negative bacteria, and no interpretation criteria were provided by both CLSI and EUCAST guidelines.[22]

For Enterobacter species, which were 100% susceptible to amoxicillin, prescribing amoxicillin and clavulanic acid is unnecessary as it may contribute to the emergence of drug-resistant strains. Recent reports have also shown an emergence ofbeta-lactamase-producing *K. pneumoniae*, which aligns with our study results.[23]

Chien et al. in 2021 concluded in their systematic review of eight studies that there are no ideal antibiotic regimens for treating orofacial infections.[24] They also stated that broad-spectrum antibiotics should not be prescribed indiscriminately, as narrow-spectrum antibiotics also offer effective results for otherwise healthy individuals.[25] In concurrence with Chien et al, our study suggests that evaluating the antibiotic resistance pattern of a patient's dental microbiota is crucial for effective antibiotic therapy and minimizing resistance development.

Pharmacologically, the selection of antibiotics should be based on their mechanism of action and the pharmacokinetic properties of the drug to ensure effective penetration into infected tissues. Beta-lactam antibiotics, such as amoxicillin and cephalosporins, act by inhibiting bacterial cell wall synthesis, making them highly effective against Gram-positive bacteria. However, resistance mechanisms, such as beta- lactamase production, have led to the need for beta-lactamase inhibitors like clavulanic acid. Macrolides, including erythromycin and azithromycin, function by inhibiting bacterial protein synthesis and are often used as alternatives in penicillin-allergic patients. Fluoroquinolones, such as ciprofloxacin, target bacterial DNA replication and have demonstrated efficacy against Gram-negative bacteria, although their use in dentistry is limited due to concerns over resistance.[26-29]

Following are the most commonly used antibiotics in dentistry:[30-32]

1. Amoxicillin: Most frequently prescribed due to broad-spectrum activity; adverse effects include
2. gastrointestinal disturbances and hypersensitivity reactions.
3. Amoxicillin-Clavulanic Acid: Effective against beta-lactamase-producing bacteria; contraindicated in patients with liver disease. Azithromycin: Used for penicillin-allergic patients; may cause QT prolongation and gastrointestinal issues.
4. Metronidazole: Effective against anaerobic infections; common side effects include nausea, metallic taste, and potential neurotoxicity.
5. Clindamycin: Alternative for penicillin-allergic patients; associated with a high risk of Clostridium difficile infection.
6. Ciprofloxacin: Used for Gram-negative infections; contraindicated in pregnant women and children due to cartilage toxicity.
7. Linezolid: Reserved for multidrug-resistant infections; may cause myelosuppression with prolonged use.

Mechanisms of Resistance:

- Beta-lactamase production – Enzymatic breakdown of beta-lactam antibiotics.
- Efflux pumps – Actively pump out antibiotics, reducing intracellular drug concentration.
- Target site mutations – Alteration of antibiotic binding sites, reducing drug efficacy.
- Biofilm formation – Creates a protective environment that limits antibiotic penetration.

Contraindications and Cost:

Amoxicillin: Contraindicated in severe renal impairment; cost-effective.
Azithromycin: Caution in cardiac patients due to QT prolongation; moderately expensive.
Metronidazole: Avoid alcohol due to disulfiram-like reaction; inexpensive.
Amoxicillin: Contraindicated in severe renal impairment; cost-effective.
Azithromycin: Caution in cardiac patients due to QT prolongation; moderately expensive. Metronidazole: Avoid alcohol due to disulfiram-like reaction; inexpensive.
Clindamycin: High risk of antibiotic-associated colitis; moderately priced. Linezolid: High cost; reserved for resistant infections.[27-30]

Pharmacotherapy of Antibiotic-Resistant Strains:

- Combination Therapy: Utilizing synergistic antibiotic combinations, such as beta- lactam antibiotics with beta-lactamase inhibitors (e.g., piperacillin-tazobactam) to combat resistant strains.
- Reserve Antibiotics: Linezolid, daptomycin, and tigecycline are used for resistant Gram-positive

- bacteria, whereas colistin and carbapenems (e.g., meropenem) are employed against resistant gram-negative bacteria
- Bacteriophage Therapy: Investigating the use of bacteriophages to target drug-resistant bacteria as an alternative treatment approach.
- Antimicrobial Peptides: Developing novel peptides that can disrupt bacterial membranes and evade resistance mechanisms.
- Adjunctive Therapies: The use of probiotics and immunomodulators to enhance host defense and reduce bacterial colonization. [33-35]

Antibiotic-resistant bacterial strains associated with dental and oral maxillofacial infections are a significant cause of morbidity and mortality worldwide and pose severe health concerns.[26-28]

Hence, patient education and public awareness about the proper use of antibiotics should be promoted to prevent misuse, such as over-the-counter drug purchases and self-medication. Additionally, implementing antibiotic stewardship programs, strict adherence to prescribing guidelines, and alternative therapeutic strategies, including adjunctive therapies like probiotics or antimicrobial peptides, should be explored in future research to combat the rising trend of antibiotic resistance effectively.

Continued research pertaining to orofacial infection of odontogenic origin with larger sample size is needed periodically as different bacterial strains emerge and the bacterial resistance to various antibiotics may vary from time to time. Advanced, rapid, feasible, cost-effective, less tedious, and technique sensitive laboratory procedures to process and to identity the bacterial strains and their sensitivity to antibiotics are required to combat emerging resistant strains.

CONCLUSION

The antimicrobial susceptibility testing revealed concerning levels of resistance to commonly prescribed antibiotics such as amoxicillin/clavulanic acid and cefuroxime, particularly among Gram-positive isolates. However, broad-spectrum antibiotics like tigecycline, carbapenems, and amikacin displayed high efficacy across all isolates, suggesting their potential role as reliable therapeutic agents in severe or resistant cases.

These findings emphasize the need for targeted antibiotic therapy informed by susceptibility testing to ensure effective treatment and to reduce the risk of antimicrobial resistance development. The wide spread resistance observed in this study also underscores the importance of judicious antibiotic use in pediatric dental cases and advocates for continuous surveillance to monitor resistance trends. Future research should focus on evaluating alternative treatment strategies and exploring novel antimicrobial agents to manage resistant infections effectively.

DECLARATIONS

Ethical statement

This study was performed in line with the principles of the Declaration of Helsinki.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CLINICAL CASE

VERTICAL PREPARATION OF TEETH IN PERIODONTAL DISEASES

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ABSTRACT

Vertical preparation techniques (VPT) have gained popularity in restorative dentistry due to their conservative approach to preserving tooth structure and improving aesthetic outcomes.

Purpose of this article is to discuss the advantages and disadvantages of the vertical tooth preparation technique, as well as to provide evidence-based recommendations for its clinical application, based on the analysis of a specific clinical case.

This study is based on a comprehensive review of relevant literature and is further supported by the detailed analysis and discussion of a specific clinical case. Conversely, when preparing teeth with intact periodontal structures, we advocate for the use of the horizontal preparation concept.

In addition, we will present the results of our own clinical research. As can be seen from the clinical case we have presented, the vertical preparation method is very effective for deep, subgingival fractures of the hard tissues of the crown part of the tooth.

This method allows to obtain the most correct marginal fit in the fracture zone and facilitate the integration of the edge of the artificial crown to the periodontal tissues. It should also be noted that we believe that the most effective use of zirconium artificial crowns is in combination with vertical preparation, since the bacteriophobic properties of zirconium contribute to faster and more adequate integration of the edge of the artificial crown to the periodontal tissues.

The vertical margin preparation technique proves to be effective when managing teeth affected by periodontal pathology. However, in cases involving intact periodontal tissues, horizontal marginal adaptation demonstrates greater clinical efficacy. We recommend the use of the vertical preparation technique in cases involving teeth with compromised periodontal support.

Keywords: parodontal deaseses, tooth preparation, horizontal preparation techniques, vertical preparation techniques (VPT)

INTRODUCTION

The development of new materials and equipment, as well as a better understanding of the biology of soft tissues, has contributed to the resurgence and evolution of a revolutionary concept in prosthodontic dentistry: preparation without shoulders.

Tooth preparation is usually performed by horizontal preparation with a clearly defined finish line or by vertical preparation (VPT) without a clearly defined finish line[1].

The preparation of the stump using horizontal finishing lines (shoulder and chamfer) has been widely practiced for the past three decades or more and has been recognized by the academic world as the gold standard of preparation[2]. Proponents of horizontal preparation argue that preparing with a shoulder helps avoid undercuts and overhanging edges of the restoration, maintains the integrity of the biological width, and simplifies the identification of the finishing line on the model by the dental technician, thereby facilitating communication between the clinician and the technical laboratory[3].

Minimally invasive methods of tooth preparation improves aesthetics and plays an important role in stress distribution, crack resistance and long-term durability [4,5].

Based on these points, the academic world has considered the concept of vertical preparation to be untenable[6]. For a long time, proponents of vertical preparation used two main techniques:

- Preparation without a shoulder, but with a bevel
- Preparation without any defined boundary.

The vertical preparation method is more conservative and defines the finish line as an area, and not as a linear border, unlike the traditional horizontal method, which often requires the removal of a significant amount of tooth tissue and can jeopardize the long-term prognosis of the tooth[7].

It is important to note that preparation without a shoulder is the most conservative method of shaping the hard tissues of the tooth and helps avoid the formation of gaps between the tooth's hard tissues and the restoration.

Preparation without any boundaries is reflected in the concepts of the Mascarella and Ignazio Loi (BOPT) schools[8]. The vertical preparation method of a full crown was first introduced by the Porta Mascarella Group in the 1980s and is defined as "periodontal" because its main indication is teeth with periodontal disease and it aims to obtain healthier periodontal tissues. The root surface that has lost periodontal attachment consists of necrotic cementum and dentin, which contains microorganisms capable of producing toxins. VEP removes unhealthy tissues, and the restoration protects the root from the oral environment. VEP is more appropriate in patients with a thick periodontal phenotype and a probing depth of more than 2 mm[9,10]. The subgingival margin of the crown will be well covered by the healed gingiva.

This approach, unlike preparation "with a bevel", involves subgingival preparation and placing the boundary of the restoration more coronally relative to the prepared boundary[11]. This method is suitable for teeth with periodontal disease but not for healthy teeth, as such preparation often results in irreversible damage to the connective tissue attachment. In the case of a healthy periodontium, this preparation is unnecessarily aggressive in the pericervical zone, as part of the prepared area remains uncovered by the restoration and is even left unpolished, leading to obvious consequences.

The proposed burs and the Mascarella School and BOPT protocols are challenging to use, especially in the absence of high magnification (microscope), requiring exceptional dexterity and skill in tooth preparation. Dentists and dental technicians typically face the following issues when applying these protocols:

- Undercuts
- Excessive taper of the prepared tooth
- Inevitable damage to the connective tissue attachment
- Profuse bleeding
- A six-week waiting period (healing phase) before taking an impression
- Unpredictable soft tissue regeneration
- Multiple relining of temporary crowns during the healing period
- Lack of certainty in determining the finish line position VPT and Periodontal Aspects[12].

the vertical preparation technique in cases involving teeth with compromised periodontal support shoes good clinical results[13-16]. Vertical preparation of the crown edge promotes the natural contour of the gum and prevents its recession.

Considering that the concept of Biological Width includes the gingival sulcus, attachment epithelium, and connective tissue attachment it is important to

emphasize, that the only structure that must not be damaged during tooth preparation is the connective tissue attachment. The attachment epithelium is merely a "connection" of epithelial structures (gingiva) with the surface (enamel) via hemidesmosomes. The epithelial structure can also be "connected" to other surfaces, such as cementum, dentin, composite material, or zirconium dioxide, under two conditions:

- The surface is smooth, hard, and clean.
- The patient does not have periodontopathy (marginal periodontitis or periodontosis).

Gingitage is essentially a curettage technique using rotating instruments (burs), performed within the sulcus or attachment epithelium zone, creating a smooth rather than torn and grooved wound surface, as seen with electrosurgery or retraction cords (when using retraction cords, additional negative factors include ischemia and tissue necrosis as a consequence). Such a wound heals through reepithelialization. The formation of a new epithelial attachment and mucosal layer, along with the return to the original tissue quality and microvascularization, occurs quickly and predictably.

The earlier definitive restoration of the tooth's hard tissues (permanent crown) is performed, the better, faster, and more predictably the regeneration and formation of new gingival epithelium will occur. This approach is borrowed from implantology: when applying the immediate loading method on an implant, the crown neck's anatomy, modeled by the dental technician, will stimulate and guide soft tissue regeneration.

Damage to the connective tissue attachment (specifically, the root cementum into which connective tissue fibers are embedded) induces an inflammatory response, leading to the release of inflammatory factors (proteases, cytokines, prostaglandins, and other molecules/enzymes), which activate osteoclasts, thereby inducing bone tissue resorption. This, in turn, increases the risk of soft tissue recession. Therefore, violations of the connective tissue attachment must be strictly avoided.

In the technique proposed by Di Febo, Loi, and others in recent years, the depth of the bur insertion into the sulcus remains quite unpredictable, highly dependent on the skills and sensitivity of the operator.

In the Vertepreparatory technique, damage to the Biological Width (BW) is virtually impossible, as the non-working end of the special bur is calibrated to avoid touching the first millimeter of the root, i.e., the area where connective tissue fibers embed into the. Furthermore, the ability to use a smaller bur tip compared to traditional vertical preparation methods allows for rotational curettage that includes only the epithelial component of the sulcus, with minimal or no bleeding and faster healing.

It is crucial that for subgingival finish lines, the restoration is designed with an adequate contour profile that will cause the gingiva to form a tight "gingival cuff" that closely fits the tooth's neck. A tight cervical cuff will protect against food impaction into the gingival sulcus and prevent the accumulation of plaque and tartar in the loose gingival tissue on the untreated tooth surface[17]. The intended contour profile is borrowed from the anatomy of the enamel profile in recently erupted teeth and its relationship with the gingival tissues, meaning it is somewhat excessive compared to conventional standards.

The technique proposed by the authors, using a bur with a non-cutting end, not only allows for better control of the degree of invasion into the gingival sulcus, but also:

- Guarantees a more conservative approach to the tooth's hard tissues.
- The final convergence of the prepared root with the adjacent tooth and the unchanged interradicular distance create better conditions for the predictable growth of the gingival papilla (see central diagram).

Predictability of gingival papilla growth.

Basis of Preparation for Vertiprep: Preparation Using a Diamond Bur with a Tapered Shape and Rounded Inert Tip

In endodontics, this bur is widely recognized as the *Batt-bur*(Figure. 1).

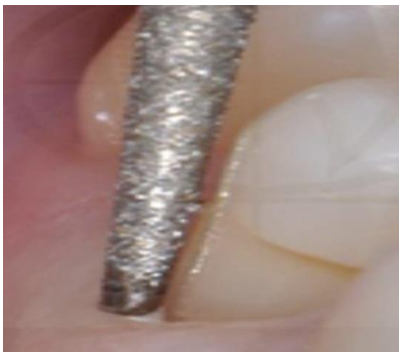


Figure 1. Batt-bur

This bur offers the following advantages:

- The conicity of approximately 2 degrees promotes the optimal taper of the stump.
- The coronal diameter of 1.2 mm and the apical diameter of only 0.7 mm minimize the reduction of the tooth's hard tissues.
- The inert, non-cutting tip of the bur, 1 mm in length, reduces or prevents damage to the connective tissue, simplifying the preparation process.
- The non-cutting tip allows for almost bloodless gingival tissue retraction, creating space for direct impression taking and easy

rebase of temporary restorations.

- The non-cutting tip enables work in the presence of a retraction cord or Teflon tape without causing damage to them.
- The bur's design helps even novice practitioners avoid undercuts.
- The non-cutting tip also allows the bur to function as a periodontal probe if necessary.
- The design of the *Batt-bur* differs from that of the flame-shaped bur used in Mascarella and BOPT approaches, enabling precise, geometric cutting of the tooth's hard tissues and thereby contributing to the creation of truly vertical finish lines.
- It facilitates immediate, seamless impression taking and temporization (fabrication of temporary crowns), unlike other approaches.

This bur is specifically designed to provide controlled and efficient tooth preparation, offering numerous benefits in terms of precision and preservation of tooth structure.

After the analytical theoretical part of the article, we find it necessary to present a clinical case of using the Vertiprep technique.

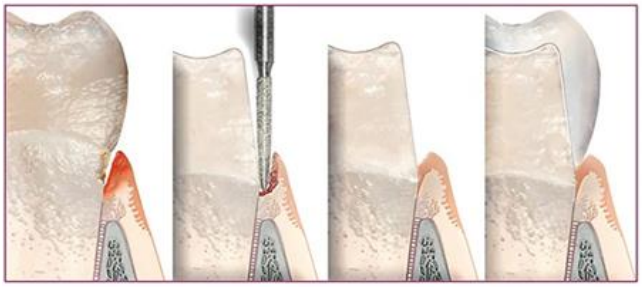


Figure 2.VEP Vertical Edgeless Preparation
Periodontal dominance in prosthetic preparation[18]

Our clinical studies and analysis of literature sources show that vertical preparation of hard dental tissues in periodontal problems not only improves the marginal fit of the tooth, but also facilitates the integration of the edge of the structure to the periodontal tissues, while saving tooth tissue.

Clinical case

A 50-year-old patient presented to us after endodontic treatment of tooth 4.6. The root canal treatment was carried out quite effectively; however, during one of the visits, the therapist accidentally fractured the medial section of the lingual wall of the coronal part of the tooth (Figure 3,4).



Figure 3, 4. Clinical situation after wall fracture

The fracture extended deeply under the gum, which led the dentist to consider tooth extraction for 4.6. The patient sought our help with the hope of saving technique.We decided to fabricate a prosthetic crown that would fit the fracture zone with a vertical finish line, while in the intact areas, we planned to create a traditional horizontal adaptation. The decision was made to manufacture the crown from zirconia oxide, given its bacteriophobic surface properties. In the intact parts of the tooth, we formed a classic chamfer margin (Figure. 5).



Figure 5. Zirconium crown with vertical fit in the fracture area

The patient has been under dynamic observation for 3 years, without complaints, and the functionality of the tooth has been fully restored.

DISCUSSION

Various tooth preparation techniques have been described,these can be generically categorized into horizontal (chamfer or shoulder) and vertical preparations (feather edge, biologically oriented preparation technique [BOPT]).[18]

Despite the various preparation designs available, clinicians often prefer the horizontal one because it provides neat margins on the initial restorations and facilitates better placement of the final restoration.

However, Agustin-Panadero et al. showed that teeth prepared using BOPT presented better periodontal outcomes in terms of gingival inflammation than those of restorations prepared using conventional horizontal finish lines. the vertical design reduces marginal gap of the restoration and creates a less irritating environment within the gingival sulcus[19].

Vertical preparation has revolutionized orthopedic dentistry and radically changed the approach to crown preparation and manufacturing. This is a new technique that became widely known about ten years ago and is now being studied and implemented in clinical practice.

Before the prosthetics of the damaged tooth, its hypersensitivity was observed, in particular, from external temperature irritants. The gums in the fracture area were swollen and hyperemic.

Dynamic clinical observation over three years shows a stable lack of response to any external stimuli. The gums in the fracture area are absolutely healthy.

The effectiveness of using the vertical preparation method in the area of the tooth fracture is obvious.

Vertical Preparation technique involves extending the subgingival preparation to ideally treat the entire exposed root surface to the epithelial attachment[20-22]. Instead of a defined finish line and associated transition angle, the technique creates a vertical tooth wall extending from the bottom of the sulcus on which the edge of the prosthetic crown can be positioned at a flexible height while still providing a secure seal, leaving the prosthetic-use area of the axial wall without corners or edges, i.e. without edges.

Main advantages of vertical preparation

- Minimally invasive in the cervical area.
- Preserves tooth structure and allows preservation of enamel in the cervical area.
- Possibility of positioning the final finishing line at different levels, either more coronal or more apical within the gingival sulcus, without affecting the quality of the marginal adaptation of the restoration.
- Possibility of modulating the emergence profile.
- Easy and quick to perform.
- Easy to take an impression.
- Easy to fabricate and finish temporary structures.

VPTs are applicable to small carious lesions below the gingival margin and are ideal for teeth with short clinical crowns, such as mandibular incisors, where horizontal preparation would result in significant loss of tooth tissue, facilitating oral hygiene and creating a natural appearance, which is especially important in the anterior regions where aesthetics are important[23-25].

VPTs disadvantages are also noticed.

- Tooth preparation is highly demanding because of the gingival sulcular involvement.
- It is very difficult to remove the excess luting cement from the gingival sulcus.
- It is also difficult to evaluate the marginal fit of the restoration

This method allowed us to avoid excessive preparation of the tooth in the fracture area and save it from extraction. It is also necessary to note the beneficial effect of zirconium on inflamed periodontal tissues, given its bacteriophobic properties.

All of the above allows us to recommend not only the vertical preparation method for periodontal problems, but also the use of zirconium prostheses.

Noè, G.et al. (2023)in their “Vertical Edgeless Preparation: Periodontal Dominance In Prosthetic Crown Preparation” sience article published in 2023 believe that the vertical preparation method is indicated for all types of periodontal pathology, as well as for short clinical crowns of abutment teeth[26].

The studies of these authors led us to the idea of the possibility of using this method in our clinical case, when the preparation contributed not only to the preservation of hard dental tissues but also to a more harmonious integration of the edge of the artificial crown to the periodontal tissues.

CONCLUSION

In conclusion of our article, we would like to recommend the method of preparing vertical finishing lines, known as Vertiprep, for patients with periodontal issues. This preparation technique is highly effective.

However, in cases involving healthy periodontium, we still recommend the traditional horizontal preparation method.

DECLARATIONS

Conflict of Interest

The author declares that he has no conflict of interest and there was no external source of funding for the present study.

Source of funding

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Informed consent

Informed consent was obtained from all individual participants included in the study.

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BULLETIN OF STOMATOLOGY AND MAXILLOFACIAL SURGERY
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ORIGINAL RESEARCH

ASSESSMENT OF RELATIONSHIP BETWEEN MAXILLARY TEETH AND MAXILLARY SINUS

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ABSTRACT

Background:The maxillary sinus is the largest paranasal sinus which has a close anatomical relationship with the roots of maxillary posterior teeth, which results in clinical challenges during dental procedures including extractions, implant procedures and endodontic treatments. Odontogenic infections and surgical interventions can lead to disruption in the sinus floor, leading to oroantral communication or sinusitis. The present study aims to evaluate the proximity of maxillary posterior tooth roots to the maxillary sinus floor using orthopantomograms (OPGs).

Methodology;A retrospective, observational study analyzing 351 OPGs of patients aged 25–30 years, were selected. The relationship between roots of maxillary posterior teeth and maxillary sinus was assessed using a modified scoring system by Sharan and Madjar (2006), which classified root projection into the maxillary sinus with a score from 0 (no contact) to 4 (>2 mm intrusion). The data were tabulated in excel sheets and statistical analysis was done using SPSS software, chi-square tests were done. A p-value of <0.05 was considered statistically significant.

Results:The present study revealed considerable variations in root-sinus proximity among different maxillary posterior teeth. The highest root projection into the sinus cavity was observed in first and second molars. Tooth 27 exhibited the most frequent intrusion into the sinus, with 47.2% scoring 3 and 22.5% scoring 4. Gender-based analysis indicated that males had a significantly higher frequency of root projection into the sinus, particularly for tooth 16 (χ^2 = 17.584, p = 0.001). Chi-square analysis demonstrated significant bilateral symmetry in root-sinus relationships, with the strongest association found between the right and left first premolars (χ^2 = 136.47, p < 0.001).

Conclusion;The study confirms the anatomical variations in the relationship between maxillary posterior teeth and the maxillary sinus floor, with molars demonstrating the closest proximity. The findings emphasize the need for thorough preoperative radiographic evaluation, particularly for procedures involving maxillary posterior teeth, to minimize complications such as sinus perforation and infections. Gender differences in sinus pneumatization and root projection suggest potential implications for personalized treatment planning.

Keywords: OPG, Maxillary sinus, Maxillary posterior teeth

INTRODUCTION

Maxillary sinus is the largest of all the paranasal sinuses, it is located bilaterally within the maxilla, and plays an important role in various physiological processes including humidification and air filtration [2]. Owing to the close anatomical proximity between the roots of the maxillary posterior teeth and the floor of the maxillary sinus, there exist significant clinical and diagnostic challenges for the clinicians while treating maxillary posterior teeth [3].

Pathological conditions which originate from the maxillary posterior teeth, such as periapical pathologies or periodontal infections, may traverse the boundaries of supporting dental structures and affect the maxillary sinus. This phenomenon is termed as odontogenic maxillary sinusitis, it is a condition which arises from dental infections (commonly dental caries) and can result in complications such as obstruction of the sinus and oroantral communication [4]. Dental procedures such as endodontic therapy or dental extractions can compromise the integrity of the sinus floor and result in conditions such as perforation of the sinus or root displacement into the sinus cavity, which may require surgical intervention [5].

The anatomy of the sinus floor is very unique, as it extends between the individual roots of adjacent teeth to form elevations which are referred to as “blocks” on the sinus antral surface [1]. Understanding the relationship between the roots of maxillary posterior teeth and maxillary sinus is essential for a dental practitioner, as it aids in treatment planning of various procedures such as implant placement, sinus augmentation, and the management of sinus-related dental pathologies and complications [6]. Various radiographic imaging modalities provide ardent information on the anatomical relationship to aid clinicians in assessing the variations in proximity to the sinus floor in order to evaluate the potential risks with the interventions. Orthopantomogram (OPG) is a valuable 2-dimensional tool which helps in visualising numerous anatomical structures of the jaws [7].

The present study aims to evaluate the relationship between the apical roots of maxillary posterior teeth and the floor of the maxillary sinus, by assessing numerous OPGs.

MATERIAL AND METHOD

Study Design

The study design is a retrospective, observational study conducted to assess the relationship between the apical roots of maxillary posterior teeth and the floor of the maxillary sinus using orthopantomograms (OPGs). Ethical clearance was obtained from the institutional ethical review board to ensure compliance with ethical standards.

Sample Selection

A total of 500 OPGs of patients who belong to the age group between 25 to 30 years visiting the Oral radiology department for diagnostic purposes were collected. Inclusion criteria was patients with intact maxillary posterior teeth with not more than two missing teeth. Patients with any form of periapical pathology involving maxillary posterior teeth, previous sinus surgeries, or dental extractions were excluded from the study. Following the above mentioned criteria, 351 OPGs were chosen eligible for further analysis.

Data Collection

The OPGs were evaluated using a modified scale with a scoring criteria proposed by Sharan and Madjar [1]. This scale categorizes the relationship between the roots of maxillary posterior teeth and the maxillary sinus floor into 5 categories ranging from 0 to 4. The radiographic assessments were conducted independently by two calibrated examiners to ensure accuracy and reliability.

Scale (scoring criteria) [8]

The scoring criteria for assessing root and sinus relationship includes a score of 0, which indicates no contact between any root of the posterior teeth and the cortical border of the floor of the sinus. Score 1 signifies that one or more posterior teeth roots are in contact with the cortical borders of the sinus. A Score of 2 indicates that one or more posterior tooth roots project laterally onto the sinus cavity, but their apices remain outside the sinus boundaries. A score of 3 indicates that one or more root apices project into the sinus cavity but less than 2 mm. Lastly, score 4 indicates that one or more root apices project into the sinus cavity by a distance greater than 2 mm.

Statistical Analysis

The data obtained were tabulated in Microsoft Excel and statistical analysis was done using SPSS software version 23. Descriptive statistics and Chi-square tests were performed to summarize and assess the significance of the relationship between each of the maxillary posterior teeth roots and the sinus floor. A p-value of <0.05 was considered statistically significant.

Ethical Considerations

Patient confidentiality was maintained by anonymizing all radiographs and records. Only the necessary data were extracted, and the study adhered to the ethical principles outlined in the Declaration of Helsinki.

RESULTS

Overview of Scoring System

The results of the study highlight the significant variability in the relationship between the roots of maxillary posterior teeth and the floor of the maxillary sinus (Table 1).

Table 1. Showing total number of teeth with respective scores

SCORE / TOOTH	0	1	2	3	4
18	69	160	11	78	33
17	18	113	13	136	71
16	11	110	15	119	96
15	34	193	20	87	17
14	148	187	4	8	4
24	180	160	4	4	3
25	59	245	19	21	7
26	22	97	25	153	54
27	13	75	18	166	79
28	40	149	8	108	46

Using a scoring system ranging from 0 to 4, the findings reveal distinct patterns of root-sinus proximity. A score of 0 indicated no contact between the root and the sinus, while scores of 3 and 4 represented roots projecting into the sinus cavity, with 4 indicating deeper penetration (>2 mm). The study focused on teeth 17, 16, 15, 14, 24, 25, 26, and 27, which are the maxillary first and second molars on both sides.

Findings for Tooth 17 and Tooth 16

For tooth 17, 38% of cases were assigned a score of 3, demonstrating root projection into the sinus cavity with less than 2 mm of separation. Additionally, 20.2% scored 4, indicating roots projecting more than 2 mm into the sinus. Similarly, for tooth 16, 27.3% of cases had a score of 3, while 33.9% exhibited a score of 4.

Findings for Tooth 26 and Tooth 27

For the left side, tooth 26 showed 43.5% of cases with a score of 3, and 15.3% with a score of 4. Tooth 27 had the highest percentage of roots projecting into the sinus cavity, with 47.2% scoring 3 and 22.5% scoring 4.

Findings for Tooth 14 and Tooth 15

Tooth 14 demonstrated the following distribution of scores: 148 cases (42.2%) scored 0, indicating no contact between the root and the maxillary sinus. A total of 187 cases (53.3%) scored 1, showing root-sinus proximity without penetration into the sinus cavity. Scores of 2, 3, and 4 were observed in 4 cases (1.1%), 8 cases (2.3%), and 4 cases (1.1%), respectively. For tooth 15, 193 cases (55.0%) scored 1, indicating root-sinus proximity without penetration. A total of 34 cases (9.7%) scored 0, showing no contact with the sinus floor. Scores of 2, 3, and 4 were observed in 20 cases (5.7%), 87 cases (24.8%), and 17 cases (4.8%), respectively

Findings for Tooth 24 and Tooth 25

Tooth 24 exhibited a distribution of scores as follows: 180 cases (51.3%) scored 0, indicating no contact with the sinus floor. A total of 160 cases (45.6%) scored 1, showing root-sinus proximity without penetration. Scores of 2, 3, and 4 were observed in 4 cases (1.1%), 4 cases (1.1%), and 3 cases (0.9%), respectively. For tooth 25, 245 cases (69.8%) scored 1, indicating root-sinus proximity without penetration, while 59 cases (16.8%) scored 0, showing no contact with the sinus floor. Scores of 2, 3, and 4 were observed in 19 cases (5.4%), 21 cases (6.0%), and 7 cases (2.0%), respectively.

Chi square test:

The chi-square test revealed significant associations between the corresponding right and left molars and premolars (Table 2). For the third molars (18 and 28), the Pearson chi-square value was 36.327 (df = 16, p = 0.003), indicating a statistically significant relationship between their anatomical or positional attributes. Similarly, the second molars (17 and 27) showed a strong association, with a chi-square value of 98.707 (df = 16, p < 0.001), suggesting a consistent bilateral relationship in their characteristics. The first molars (16 and 26) exhibited an even stronger association, with a chi-square value of 113.685 (df = 16, p < 0.001), underscoring their bilateral symmetry or shared morphological patterns (Figure 1).

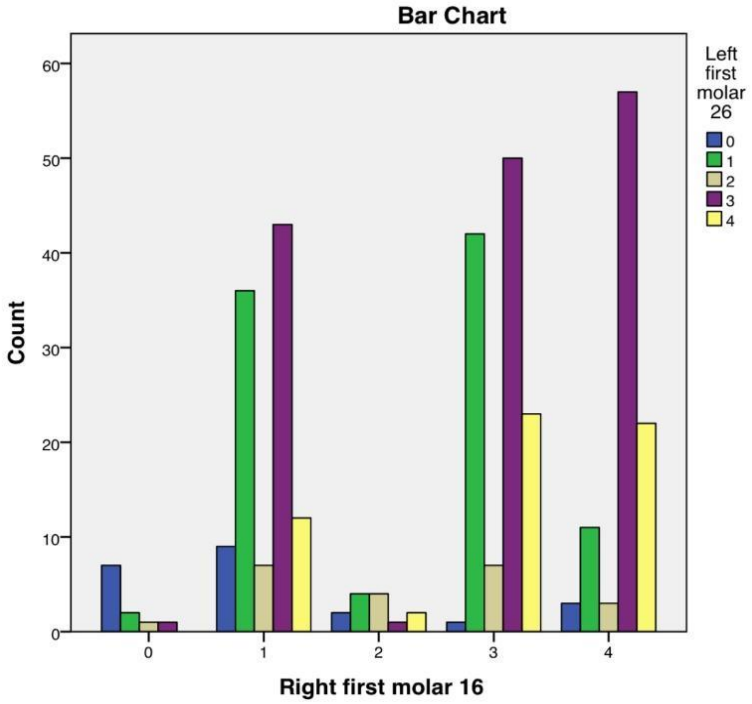


Figure 1. Graphical representation of the correlation of the relationship of maxillary sinus to right and left first maxillary molar

The second premolars (15 and 25) also demonstrated a highly significant relationship, with a chi-square value of 116.444 (df = 16, p < 0.001). Lastly, the first premolars (14 and 24) displayed the strongest bilateral association among the tested groups, with a chi-square value of 136.470 (df = 16, p < 0.001).

The second premolars (15 and 25) also demonstrated a highly significant relationship, with a chi-square value of 116.444 (df = 16, p < 0.001). Lastly, the first premolars (14 and 24) displayed the strongest bilateral association among the tested groups, with a chi-square value of 136.470 (df = 16, p < 0.001).

Gender-Based Analysis

The gender-based analysis indicated variations in root-sinus proximity for certain teeth, particularly tooth 16 (Table 3).

Table 2. showing Chi square test results between right and left posterior teeth

Teeth Pair	Chi-Square Value	p-Value
Right Third Molar (18) - Left Third Molar (28)	36.327	0.003
Right Second Molar (17) - Left Second Molar (27)	98.707	<0.001
Right First Molar (16) - Left First Molar (26)	113.685	<0.001
Right Second Premolar (15) - Left Second Premolar (25)	116.444	<0.001
Right First Premolar (14) - Left First Premolar (24)	136.47	<0.001

Table 3 showing Chi square test results for gender based analysis

Teeth	Chi-Square Value	p-Value
Right Third Molar (18)	4.908	0.297
Right Second Molar (17)	2.714	0.607
Right First Molar (16)	17.584	0.001
Right Second Premolar (15)	0.606	0.962
Right First Premolar (14)	6.052	0.195
Left First Premolar (24)	11.916	0.018
Left Second Premolar (25)	10.962	0.027
Left First Molar (26)	0.611	0.962
Left Second Molar (27)	3.794	0.435
Left Third Molar (28)	3.934	0.415

Male patients accounted for a higher percentage of cases with scores 3 and 4 compared to females. Chi square tests for association between gender and the positional or anatomical characteristics of molars and premolars showed the following results. Right third molar (18), the chi-square test revealed no significant association with gender ($\chi^2 = 4.908$, df = 4, p = 0.297). Similarly, the right second molar (17) ($\chi^2 = 2.714$, df = 4, p = 0.607) and the right second premolar (15) ($\chi^2 = 0.606$, df = 4, p = 0.962) showed no significant gender-based differences. However, a significant association was observed for the right first molar (16) ($\chi^2 = 17.584$, df = 4, p = 0.001), suggesting potential gender-related differences in its positional attributes (Figure 2).

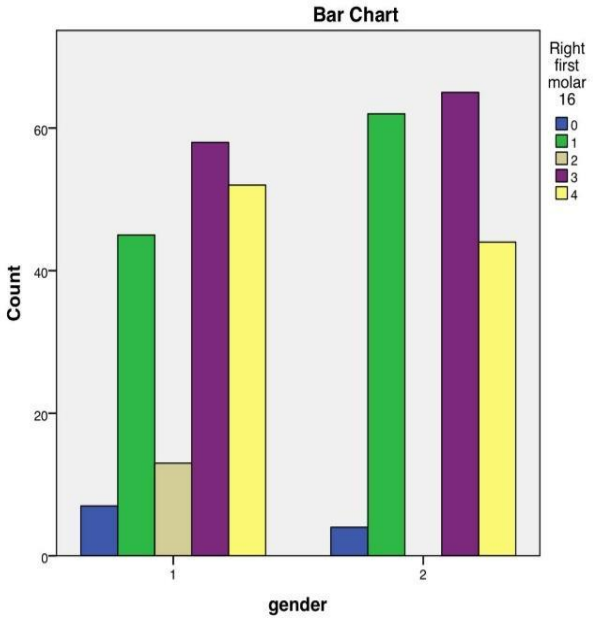


Figure 2. Graphical representation of the correlation of gender and the relation of maxillary sinus to right first maxillary molar

On the left side, the first premolar (24) ($\chi^2 = 11.916$, df = 4, p = 0.018) and second premolar (25) ($\chi^2 = 10.962$, df = 4, p = 0.027) also showed significant associations with gender, while other teeth, including the left first molar (26) (Figure 3), left second molar (27), and left third molar (28), did not exhibit any significant gender-based differences (p > 0.05).

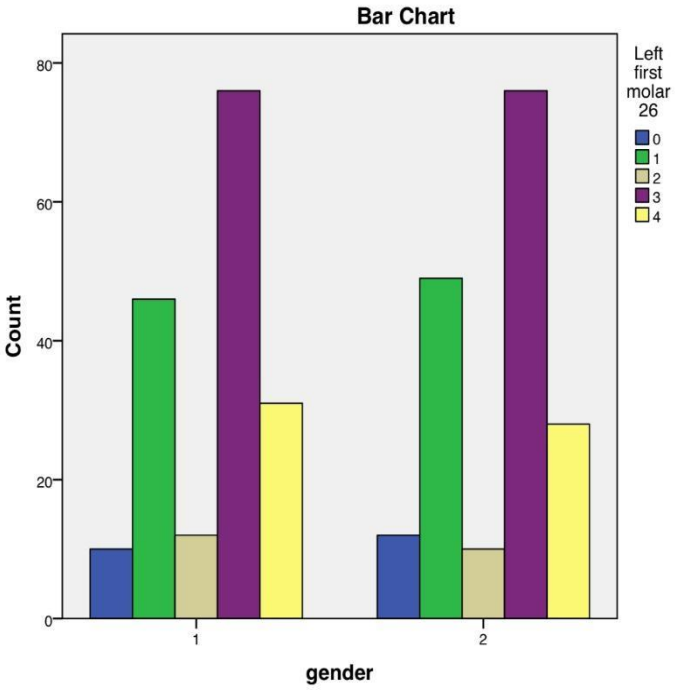


Figure 3: Graphical representation of the correlation of gender and the relation of maxillary sinus to left first maxillary molar

DISCUSSION

The anatomical relationship between the maxillary sinus and the roots of posterior maxillary teeth plays a critical role in numerous dental and surgical procedures. The present study evaluated the relationship between maxillary tooth roots and their proximity to the sinus floor.

Variations in the anatomy of the maxillary sinus are influenced by several factors such as gender, age, and oral health [9]. In the present study, gender based variations in proximity of the posterior teeth roots to the floor of the sinus are noted, especially in tooth 16, which exhibited significant proximity to the sinus compared to other teeth. This could be due to differences in craniofacial morphology among males and females, wherein males usually demonstrate larger maxillary sinuses with thicker alveolar bones compared to females, with increased pneumatization of sinus leading to thinner alveolar bone which separates the roots and the sinus floor [10].

Variations in sinus root proximity have been observed among various populations which highlight the influence of ethnicity and geographical factors [11]. Also genetic predisposition, dietary habits greatly influence jaw development and play an ardent role in such differences [12]. The findings observed in the present study reveal that gender based craniofacial patterns, sinus dimensions and density of alveolar bone contribute to the varied anatomic relationships observed in root proximity to the sinus.

Literature states that sinus pneumatization is an age-related phenomenon which influences root proximity. It has been observed that the pneumatization process continues till the second decade in females and third decade in males [13]. The early skeletal maturation in females compared to males contributes to gender based variations in root proximity, as observed in the present study.

Among the first and second premolars, 14, 15, 24 & 25, and the anterior teeth, minimal sinus involvement is reported [14]. These findings are concordant with the present study as predominantly lower scores were observed among the premolars depicting minimal interaction. The reason could be due to short roots and more anterior positioning of such teeth. Also, there is a presence of denser bone in the anterior maxilla which serves as a structural barrier between the roots and sinus [15].

Sharan et al. observed that the roots of the maxillary first and second molar are in close proximity to the sinus floor often with their roots projecting into the sinus cavity [8]. These findings are concordant with the findings observed in the present study. The reason for close proximity in molars could be due to age-driven pneumatization of the posterior maxilla which can be further exacerbated by tooth loss. The development of mesiobuccal and palatal roots of molars coincides along with sinus expansion, which ultimately results in reduced bony separation between these roots and sinus [16].

A study by SH et al. revealed that the mesiobuccal root of the maxillary first molar often lies in closest proximity to the sinus floor, emphasising the need for precise imaging prior to treatment planning [17]. These findings are similar to the findings observed in the current study. The reason for close proximity among the mesiobuccal root of the first molar to the sinus floor could be due to its developmental positioning which increases the risk of direct sinus contact [18].

Studies conducted using 3-dimensional modalities such as CBCT reveal that first molars are in direct contact with their root apices projecting into the sinus cavity [19]. These findings support the results observed in the present study. Since maxillary molars are associated with narrow alveolar bone width, in areas of significant pneumatization, along with a widely spread root morphology, increasing its likelihood of interaction with the sinus cavity [20].

The buccodistal root of the second molars has been reported to have close proximity to the sinus in a few studies [21], which aligns with the findings of the present study. The buccodistal root of the second molars are located in regions with the thinnest alveolar bone density and also owing to the curvature of the maxillary arch, this region is exposed to increased mechanical stresses and bone resorption, thereby reducing its thickness further [16]. The findings for teeth 17 and 26 in this study reinforce the need for detailed imaging and surgical planning.

Studies indicate that the thickness of Schneiderian membrane varies depending on the underlying pathology. Teeth with higher proximity scores such as 3 and 4 as seen in first and second molars, are often associated with greater risks of sinus perforation during surgical interventions [20]. The findings for teeth 17 and 26 in this study reinforce the need for detailed imaging and surgical planning. In instances such as chronic inflammation and anatomical variations, thinning of the Schniedarian membrane can occur. When the apices of posterior teeth roots are in close proximity, it further weakens the membrane and increases the chances of perforation during surgery [22].

Studies on sinonasal complications of dental disease and treatment (SCDDT) underscore the significant role of dental pathology in sinusitis. The anatomical proximity of maxillary posterior tooth roots to the sinus floor can contribute to either unilateral or bilateral sinus inflammation. These findings highlight the necessity of precise diagnostic imaging, such as CT scans, and a multidisciplinary approach for effective management [23].

Studies on sinus septa reveal the anatomical variations in the maxillary sinus and its proximity to the posterior teeth. The first and second molar regions demonstrated highest prevalence of septa, which can lead to complications in sinus lift procedures, thus reinforcing the importance of detailed evaluation of sinus-tooth proximity to minimize surgical risks [24].

Studies have shown that there exist variations in sinus depths with shallow regions located between the right maxillary molar and deeper regions between the left maxillary premolars [25]. These findings are in concordance with the present study where the majority of the premolar teeth exhibited low proximity scores (0 or 1), indicating a more distant anatomical relationship with the sinus floor. Age and gender also played a role, with older individuals showing greater root-sinus separation and males exhibiting thicker buccal bone than females [26, 27]. Similar trends were observed in the present study.

Clinical Implications

This study underscores the importance of comprehensive radiographic assessments, particularly for posterior teeth, where scores of 3 or 4 indicate a high risk of complications such as sinus perforation or oroantral communication. Teeth 16, 17, 26, and 27, which demonstrated significant proximity to the sinus floor, require meticulous planning for procedures such as extractions, implants, or endodontic therapy.

While anterior teeth, such as 24 and 25, exhibit minimal sinus interaction, anatomical variability necessitates thorough evaluations even for these teeth to minimize potential risks.

Future Directions

Future research should investigate factors such as age-related changes, population-based anatomical variations, and sinus septa morphology to provide a more comprehensive understanding of the root-sinus relationship. Incorporating advanced imaging modalities, such as CBCT, into routine evaluations will enhance diagnostic accuracy and improve treatment outcomes.

CONCLUSION

The findings of this study align with existing literature, emphasizing the importance of understanding anatomical variations and using detailed imaging for safe and effective dental procedures. Further research into ethnicity and age-related anatomical changes could offer additional insights into the relationship between maxillary teeth and the sinus floor.

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Consent for publication
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None

Competing interest
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