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ԲԱՆԲԵՐ ՍՏՈՄԱՏՈԼՈԳԻԱՅԻ ԵՎ ԴԻՄԱԾՆՈՏԱՅԻՆ ՎԻՐԱԲՈՒԺՈՒԹՅԱՆ
Գիտագործնական հանդես

Գլխավոր խմբագիր - Հակոբյան Գ.Վ.
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REVIEW ARTICLE

CORTICOSTEROIDS IN IMPACTED MANDIBULAR THIRD MOLAR SURGERY: A NARRATIVE REVIEW

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ABSTRACT

Aim: To systematically evaluate the clinical efficacy, optimal dosage, route, timing, and safety of corticosteroid administration in managing postoperative complications—pain, swelling, and trismus—following impacted mandibular third molar surgery.

Materials and Methods: PubMed, Scopus, Web of Science, and Google Scholar were searched using a pre-specified search strategy. Clinical studies, reviews, systematic reviews, case reports, and case series were included for data synthesis.Search keywords terms included**:** Corticosteroids, Mandibular Third Molar, Impacted Tooth, Dental Surgery, Postoperative Complications, Swelling, Pain Management, Trismus, Anti-inflammatory Drugs.

Results:A total of **315** titles were screened rigorously by two independent evaluators. After duplicate exclusion and removal of irrelevant titles, 87 articles were included for full-text analysis, out of which **35** qualified for final data synthesis.

Conclusion: Corticosteroids especially dexamethasone demonstrate significant effectiveness in reducing postoperative complications when administered via intravenous, intramuscular, or submucosal routes either preoperatively or intraoperatively. While oral administration is preferred by patients for its convenience, its efficacy is comparatively lower. Standardized clinical protocols and individualized patient assessment are essential to maximize therapeutic outcomes while minimizing potential adverse effects.

Keywords: Corticosteroids, Mandibular Third Molar, Impacted Tooth, Dental Surgery, Postoperative Complications, Swelling, Pain Management, Trismus, Anti-inflammatory Drugs

INTRODUCTION

The extraction of impacted mandibular third molars stands as a frequent procedure within oral and maxillofacial surgery while leading to major postoperative complications involving pain together with facial swelling and trismus. [1-4] The complications create adverse effects which harm patient life quality while extending recovery time and creating a continuous clinical problem. [3,4] The exact severity of postoperative complications depends on surgical trauma extent while also considering how patients handle surgery and the selected techniques. [5]

Different pharmaceutical interventions such as analgesics alongside anti-inflammatory drugs and antibiotics and corticosteroids are used to manage the complications after surgery. [6] The administration of corticosteroids continues to gain traction in medical practice because these medications provide strong anti-inflammatory effects that control surgical trauma-induced inflammatory responses. [7,8] Corticosteroids block the activity of phospholipase A2 enzyme to halt arachidonic acid synthesis which leads to decreased prostaglandin and leukotriene production for inhibition of inflammatory response signs like swelling and pain. [9,10]

Widespread acceptance of corticosteroids for third molar surgery has led to inconsistent clinical findings because researchers use diverse methods of drug delivery (oral, intravenous, intramuscular, submucosal) with different dosage schedules and timing protocols and corticosteroid types. [11,12] The evaluation of present evidence needs to be extensive so healthcare professionals can identify the best practices for corticosteroid management in mandibular third molar procedures to achieve uniform treatment benefits and reduce side effects.

The oral and maxillofacial surgery field has provided comprehensive research on four corticosteroids:

dexamethasone, methylprednisolone, betamethasone, and prednisone because of their effective treatment and beneficial safety characteristics.[13] Medical practitioners use dexamethasone as their primary corticosteroid because it exhibits strong anti-inflammatory properties with an extended half-life and weak mineralocorticoid activity along with multiple administration options. [14] Dexamethasone demonstrates proven effectiveness to decrease both postoperative edema and pain when given before or during surgery which makes it a preferred treatment method in third molar procedures. [8,15]

Research continues to challenge the best practices for corticosteroid usage and when to administer them despite their broad established beneficial properties. Clinical efficacy of corticosteroids before surgery receives support from various studies but researchers disagree about the proper timing and appropriate dosing route. [16] Dexamethasone utilized as a single pre-surgical dose demonstrates superior effectiveness for reducing postoperative complications than administering it after surgical procedures have begun. [12] Studies have shown similar outcomes for corticosteroid benefit when administered before operation and right after operation thus requiring established treatment guidelines founded on sound clinical proof.[17]

Patients receiving corticosteroids need close monitoring due to possible adverse effects which include delayed wound healing and higher infection risk and blood sugar changes in addition to digestive problems. [18] The administration of short-term corticosteroids during third molar surgery has proven to be safe according to studies even though other researchers have certain reservations. [19] Healthcare professionals should weigh advantages against dangers while providing personalized care to their patients through proper consent protocols and proven clinical practices. [20]

The objective of this review is to systematically evaluate published studies regarding clinical outcomes of corticosteroid utilization and technical protocols along with dosage schedules and timing protocols and side effects in surgeries involving impacted mandibular third molars with evidence-based practices for medical practitioners.

MATERIAL AND METHODS

PubMed, Scopus, Web of Science, and Google Scholar were searched using a pre-specified search strategy. Clinical studies, reviews, systematic reviews, case reports, and case series were included for data synthesis.

The review was conducted according to the PRISMA guidelines (PRISMA flow chart is presented in figure1.)

A total of **315** titles were screened rigorously by two independent evaluators. After duplicate exclusion and removal of irrelevant titles, 87 articles were included for full-text analysis, out of which **35** qualified for final data synthesis.

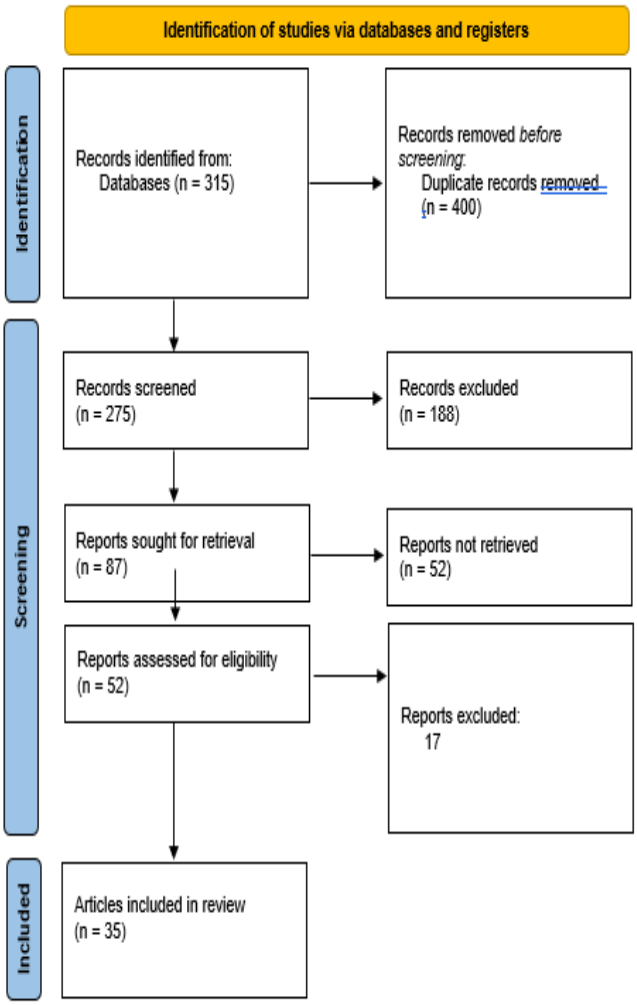


Figure 1. PRISMA flow chart

Patients who get surgical removal of buried mandibular third molars typically experience pain alongside swelling and trismus which strongly impacts their daily life quality. Intranasal corticosteroids have gained recognition as an effective treatment solution because they provide strong anti-inflammatory effects. A critical analysis evaluates the clinical impact and recommended dosages and administration routes for corticosteroids being used throughout impacted mandibular third molar surgery. The review also examines the timing of administration and safety considerations of these medications. Strong scientific findings from the analyzed research demonstrate that corticosteroids effectively decrease the complications following surgical procedures. Standardized clinical guidelines together with individual patient management approaches are necessary due to the existing protocol variations.

Mandibular third molar extractions when impacted function as one of the most common procedures in oral surgery and generate complications like pain alongside facial swelling and trismus which degrade recovery after surgery. [1,2] The postoperative complications impact patient quality of life strongly so various medications have been introduced to reduce these complications. [3] The clinical applications of corticosteroids became widespread because these medications demonstrate both strong anti-inflammatory effects and immunosuppressive actions. [4,5] The pharmacological effects entail a reduction of phospholipase A2 activity together with the subsequently lower levels of pain-causing prostaglandins and leukotrienes.[6]

Research has demonstrated corticosteroids' effectiveness but differences exist in the selection of optimal corticosteroid as well as dosage and administration route and timing.[7]

This examination evaluates existing studies about corticosteroids and recommends suitable protocols to reduce postoperative effects of impacted mandibular third molar surgery.

Clinical Efficacy of Corticosteroids

Clinical studies have extensively evaluated the oral surgery performance of dexamethasone together with methylprednisolone betamethasone and prednisone. [8] Current research supports dexamethasone as the most effective corticosteroid since it achieves strong anti-inflammatory reactions and demonstrated a longer biological half-life while having low mineralocorticoid potential along with simple administration via different delivery routes. [9] Dexamethasone reduces all three postoperative side effects including swelling and pain and trismus than placebo. [10] Dexamethasone provided superior results for treating postoperative inflammation over hydrocortisone and methylprednisolone. [11]

Routes of Administration

Administration of corticosteroids can occur through different routes such as oral, intravenous, intramuscular as well as submucosal delivery with their own unique advantages and drawbacks. [12] Postoperative symptom management improves through IV and IM drug administration which ensures quick systemic absorption along with enhanced bioavailability. [13] Since oral corticosteroids undergo first-pass metabolism this makes their potency weaker but they remain advantageous because their administration is easy and patients have better compliance while avoiding injection complications. [14] Submucosal injections deliver their anti-inflammatory drugs directly to the local area where they reduce edema and trismus while causing low systemic absorption. [15] The route selection depends on surgical complexity together with patient-specific needs and requires clinical expertise. [16]

Timing and Dosage

Postoperative results depend heavily on the method when corticosteroids are given in addition to how much is administrated. Preoperative corticosteroid administration demonstrates better effectiveness than postoperative treatment because it stops inflammatory mediators before surgery. [17] Dexamethasone become more effective for controlling pain and swelling before surgery instead of after surgery. [18] Postoperative administration of drugs at the moment of surgery leads to equal clinical advantages as reported by previous studies. [19] Recent studies have revealed that dexamethasone doses between 4 to 8 mg effectively treat patients with minimal side effects thus making them optimal for routine clinical use. [20]

Safety and Adverse Effects

Although corticosteroids show good safety when used temporarily certain side effects such as delayed wound healing and elevated infection risk and gastrointestinal symptoms and brief hyperglycemia require attention. [21] Properly administered short-term corticosteroid treatment in third molar surgery produces minimal severe side effects. [22] Medical practitioners need to thoroughly review both the patient's medical records and their systemic health alongside evaluating treatment potential risks prior to giving corticosteroid prescriptions. [23] Healthcare providers must practice patient-specific care through consent procedures and continuous postoperative checks to protect patients from complications. [24]

Patient Selection and Contraindications

A proper selection of patients becomes priorities for corticosteroid treatment in mandibular third molar surgery. Medical professionals need to evaluate each patient's health background due to corticosteroid effects on conditions such as diabetes mellitus, hypertension, peptic ulcers, immunosuppression and known allergies. [25] Diabetic patients treated with short-term corticosteroids experience temporary hyperglycemia therefore patients must receive detailed monitoring and diabetic management needs adjustment. [26] Medically vulnerable people with active infections or compromized immune systems should receive corticosteroids carefully because their use could worsen infection risks while delaying healing processes. [27]

Combination with Other Pharmacological Agents

Research indicates that the simultaneous use of corticosteroids with NSAIDs and analgesics improves control of symptoms after third molar extractions. [28] When NSAIDs are combined with corticosteroids the drugs amplify their pain-reducing effects alongside their anti-inflammatory action to boost postoperative comfort and life quality. [29] Medical providers must consider both advantages and enhanced gastrointestinal risk factors and bleeding susceptibility before prescribing these medication combinations to patients with gastric or bleeding conditions. [30]

Impact on Quality of Life and Patient Satisfaction

Patient Quality of Life together with Satisfaction Rates receive direct effects from surgical care.

Patient satisfaction together with adherence levels directly depend on postoperative outcomes which determine the overall quality of life. Research studies have proved that patients who receive corticosteroids show better reported outcomes because these drugs minimize pain alongside swelling and functional limitation which allows patients to resume their regular activities faster. [31,32] Research indicates that better postoperative comfort leads to increased patient satisfaction together with higher acceptance of surgical treatment thus showing strong clinical and psychological significance of quality postoperative care. [33]

Limitations of Current Evidence and Recommendations for Research

A substantial amount of research establishes corticosteroid effectiveness during third molar surgeries but great differences between research methods and corticosteroid dosing and administration routes and outcome measures prevent broad generalization of study findings.[34] Additional well-designed randomized controlled trials together with established outcome criteria and complete safety checks will help advance clinical guidelines. Research on individualized treatment methods should connect specific therapeutic protocols to patient risk factors and surgical intricacy to achieve maximum therapeutic results and safety standards.[35] (Table 1).

Table 1. Summary of the papers included

S.No	First Author	Year	Key Findings
1	Singh	2023	Preemptive dexamethasone better than methylprednisolone
2	Priyanga	2022	Sublingual dexamethasone effective for swelling and trismus
3	Alcântara	2014	Preemptive corticosteroids reduce pain and swelling
4	Martins-de-Barros	2021	Dexamethasone superior to ketorolac
5	Zandi	2008	Steroids more effective than rubber drains
6	Firoozi	2022	Kinesio taping can reduce sequelae
7	Nehme	2021	Piezosurgery + IM dexamethasone lowers pain
8	Parhizkar	2022	Corticosteroids improve patient outcomes
9	Costa	2023	Blood concentrates affect inflammation signs
10	Laureano Filho	2008	Higher dose dexamethasone reduces edema
11	Lima	2017	Oral dexamethasone better than diclofenac
12	Chen	2017	Submucosal dexamethasone reduces discomfort
13	Gholami	2021	IM methylprednisolone relieves swelling, pain
14	O'Hare	2019	Submucosal dexamethasone shows efficacy
15	Shoohanizad	2020	Pre-op dexamethasone more effective than post-op
16	Lacerda-Santos	2023	Photobiomodulation reduces trismus and edema
17	Ngeow	2016	Corticosteroids remain useful in third molar surgery
18	Feslihan	2019	Photobiomodulation may replace steroids
19	Mijailovic	2023	Dexamethasone better than etoricoxib
20	Gonçalves	2022	Tramadol injection shows limited benefit
22	Koray	2014	HA spray can improve symptoms post-surgery

23	Lima TC	2018	Dexamethasone+NSAID+codeine effective
24	Barbalho	2017	Dexamethasone+nimesulide improves comfort
25	Almadhoon	2022	Route and dose influence dexamethasone outcome
26	Almeida	2019	Steroids superior to placebo
27	La Rosa	2024	Etoricoxib effective both pre/post-op
28	Antunes	2011	IV vs IM dexamethasone efficacy similar
29	Falci	2017	Preemptive dexamethasone reduces sequelae
30	Chugh	2018	Submucosal vs IM corticosteroids effective
31	De Marco	2021	Flap design influences pain/swelling
32	Caymaz	2019	PRF variants improve healing outcomes
33	Azab	2022	Secondary closure reduces complications
34	Brignardello	2012	Adjuvant laser therapy moderately effective
35	UStün	2003	Methylprednisolone dosage impacts results

Future Directions

The future of research needs to develop standardized procedures for prescribing corticosteroids during impacted mandibular third molar surgeries including steroid selection and dosing amounts along with administration methods and application times. To validate current evidence researchers need to execute new large-scale randomized controlled trials together with additional meta-analyses that generate specific treatment recommendations.

CONCLUSION

A successful application of corticosteroids minimizes adverse effects which occur after impacted mandibular third molar surgery. Professional recommendations favor dexamethasone as the most appropriate corticosteroid because of its effectiveness and safety parameters but specific optimization of dosage schedule combined with delivery method is essential. Following standardized guidelines alongside improved patient selection process along with evidence-based protocols delivery will result in better therapeutic outcomes and patient satisfaction.

DECLARATIONS

The author declares no conflicts of interest and confirms that this review is based on previously published studies and does not involve any new studies with human participants or animals performed by the author.

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None

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REVIEW ARTICLE

ANTIBIOTIC PROPHYLAXIS FOR PREVENTION OF POSTOPERATIVE INFECTIONS FOLLOWING IMPACTED TOOTH REMOVAL: A COMPREHENSIVE REVIEW

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Abstract

Numerous controversies exist about the use of antibiotics for third molar extraction which stands as a prevalent oral surgical intervention. The review analyzes present data about antibiotic preventative measures following procedures involving impacted teeth extraction. Randomized trials combined with systematic reviews support that providing antibiotics before treatment reduces infection possibilities between 34% and 66%. Patients require antibiotic prophylaxis when their surgery involves deep impactions and belongs to older age groups with lengthy procedures including complex surgeries. Amoxicillin along with its combination form amoxicillin/clavulanic acid continue to be the preferred antibiotic medications but they present unique advantages and disadvantages. No research-based findings indicate that healthy patients need prophylactic medications before having uncomplicated extractions. Medical practitioners should maintain a balance between protecting patients from infection while managing antibiotic usage and evaluate individual patient characteristics for determining appropriate preventive therapies.

Keywords: Antibiotic prophylaxis; Third molar extraction; Impacted tooth; Surgical site infection; Dry socket; Postoperative complications

INTRODUCTION

The removal of third molars stands as one of the most common procedures within oral and maxillofacial surgery since healthcare providers perform millions of such operations yearly throughout the world[1]. The procedure has become common but patients face substantial dangers from postoperative consequences which include abscess, dry socket and infection alongside discomfort and swelling and limited mouth opening [2]. Surgical site infections after third molar surgery are reported to affect 0.4% to 6% of patients while dry socket develops in 1% to 30% of patients according to medical studies [3, 4].

Professional practitioners commonly prescribe prophylactic antibiotics because of these possible complications. The practice remains controversial among professionals because there exists no agreement regarding prophylactic antibiotic usage for third molar extractions[5]. The disagreement about antibiotic prescriptions during third molar extractions exists due to research contradictions alongside antibiotic resistance concerns alongside adverse effects and cost efficiency considerations [6].

According to Peterson the main requirements for antibiotic prophylaxis include selecting surgical procedures with high infection risk and choosing appropriate antibiotics and proper dosage levels and correct timing and using minimal effective treatment duration [7]. The choice to provide antibiotics before third molar extractions remains contested among professionals when treating healthy patients.

Research trying to establish antibiotic prophylaxis effectiveness in third molar surgery produced inconclusive results due to methodological issues and outcome definition heterogeneity in primary studies [8,9]. The Cochrane review noted that antibiotic use could help prevent infections yet suggested that benefits from this practice might not justify such risks because 19 patients require treatment to stop one infection based on the available evidence [4].

The present study reviews recent evidence about antibiotic prophylaxis for surgical site infections after impacted tooth surgery while exploring risk factors for treatment and analyzing different antibiotic protocols

with clinical practice recommendations.

2. Types of Postoperative Infections Following Third Molar Extraction

2.1 Surgical Site Infection

The most common signs of Surgical site infections (SSIs) after third molar extraction include pain alongside swelling, purulent discharge, abscess development and cellulitis[9]. Data shows that SSIs occur between 0.4% and 6% of patients although bone removal procedures show higher incidence [3,9].

The temporal onset of surgical site infections divides them into normal and delayed types where early-onset forms occur during the first seven days and delayed-onset develops between 2 to 8 weeks postoperatively[10]. Early bacterial contamination through surgical procedures leads to early-onset infections whereas delayed-onset infections emerge from bacterial persistence which affects either the socket or surrounding tissue areas mainly affecting patients with restricted distal space[10].

The majority of postoperative infections develop from regular oral microorganisms which include both aerobic and anaerobic bacteria. The main pathogens responsible for postoperative infections comprise streptococci, staphylococci and bacteroides and fusobacterium which belong to gram-negative bacilli [8].

2.2 Dry Socket (Alveolar Osteitis)

Dry socket develops as alveolar osteitis which produces intense pain alongside total blood clot destruction in the extraction site during the postoperative day 1-3 period [4]. Dry socket serves as localized osteomyelitis but stands outside the true infection category because researchers include it in antibiotic prophylaxis studies [11].

Recent research about dry socket incidence after third molar extractions presents conflicting information that ranges between 1% and 30%[12]. The list of known risk elements consists of smoking combined with oral contraceptive use yet also contains poor oral hygiene practice alongside traumatic extractions and difficult impactions [12].

2.3 Other Infectious Complications

Other less common infectious complications include osteomyelitis, fascial space infections, and rarely, life-threatening infections such as Ludwig's angina or cavernous sinus thrombosis [3]. These severe complications are more likely to occur in immunocompromised patients or when infections are inadequately treated [3].

3. Risk Factors for Postoperative Infections

3.1 Patient-Related Factors

Multiple characteristics which belong to patients have been identified as risk factors for developing postoperative infections. Recent research proves that patients beyond the age of 35 tend to develop more infections at higher frequencies[13]. Research data showed that patients above 36 years of age developed more postoperative infections compared to their adult counterparts[13].

The risk of microbial infections elevates when patients present with diabetes mellitus alongside HIV/AIDS and when they use immunosuppressive drugs and experience malnutrition complications [3]. Most antibiotic prophylaxis clinical trials decided against including medically needy patients even though there exists insufficient research about appropriate preventive measures for such vulnerable groups [4].

Patient-based factors that increase susceptibility to infection include smoking combined with inadequate oral hygiene and hormonal states during pregnancy and oral contraceptive use [12].

3.2 Anatomical Factors

Anatomical characteristics of the impacted tooth significantly influence infection risk. The depth of impaction, as classified by Pell and Gregory or Winter's classification, correlates with postoperative infection rates [9,13]. Fully impacted teeth requiring significant bone removal present higher risks compared to partially erupted teeth [14].

Tooth angulation also affects infection risk, with mesioangular and horizontal impactions generally requiring more extensive surgical manipulation and thus carrying higher infection risks [9].

3.3 Surgical Factors

The length of surgical procedures strongly determines the risk of infections developing. Postoperative infections grow in direct proportion to surgical time which serves as a reliable predictor for infection development[13]. The length of surgical procedures leads to increased tissue handling and bacterial contamination together with surgical complexity which results in higher infection rates.

Various studies [9,13] have confirmed that intraoperative hemostatic procedures present substantial risks for developing postoperative infections.

The infection risk during surgery depends on the procedures which the surgeon performs especially regarding flap designs and bone removal procedures as well as techniques used for wound closure [14]. The educational attainment of surgeons proved to be connected to infection rate ($p=0.021$, odds ratio=0.14) thus demonstrating that surgical proficiency directly affects surgical outcome [14].

4. Antibiotic Prophylaxis: Current Practices

4.1 Types of Antibiotics Used

The prophylaxis treatment of third molar surgery requires evaluation of multiple antibiotics while penicillins remain the standard choice for practice. Medical professionals prescribe amoxicillin alone or in combination with clavulanic acid as the primary antibiotic due to its wide antipathogenic properties and desirable drug properties

and acceptable safety profile [15].

Patients who are allergic to penicillin can receive their prophylaxis from three medications: clindamycin, azithromycin and metronidazole [7,8]. These anti-infectives show unique attributes that affect their use as well as drawbacks in different applications. Research has demonstrated that Clindamycin effectively treats oral infection anaerobes through proven concentrations of 150mg, 300mg, and 600mg [7]. Single-dose treatment with extended tissue distribution becomes possible because azithromycin has a half-life span of 8 days [8].

4.2 Timing and Duration

Provisional timing of antibiotic administration determines the success rate of prophylactic measures. Doctors typically advise patients to take antibiotics within one hour prior to surgery for adequate tissue presence during the medical operation [11]. Antibiotic levels need to remain high during the period of possible bacterial contamination according to Peterson's principle [7].

Medical research studies together with clinical practice employ numerous time periods for antibiotic prophylaxis administration. True prophylaxis consists of one preoperative dose only yet multiple medical practitioners provide antibiotic coverage lasting between 3 to 7 days after surgery [1,6]. Medical experts describe these longer antibiotic treatments as empiric therapy since they avoid the definition of true prophylaxis [3].

Medical evidence indicates that preoperative antibiotics administered at a single dose produce similar results to longer administration periods. The administration of a single 500 mg dose of azithromycin conducted better than using multi-day regimens since it led to a 2% (8) infection rate after surgery. No clear evidence shows that prolonged antibiotic usage beyond 3 days offers benefits to patients yet extends the risks of side effects and antibiotic resistance [15].

4.3 Administration Routes

Systemic antibiotics applied as oral plans or intravenous treatments remain the main method for surgical site prevention [1]. The two approaches work toward reaching adequate antibiotic distribution throughout surgical site blood and tissues.Researchers have studied topical antibiotics because they could be suitable alternatives or joint treatments alongside systemic prophylaxis.

The application of tetracycline ointment on the tongue's dorsum once every six hours after surgery for two days resulted in lower oral cancer surgery site infections than control treatments (19.6% vs. 36.1%) [2]. Research about prophylaxis for third molar extractions remains scarce.

5. Evidence for Efficacy of Antibiotic Prophylaxis

5.1 Effect on Surgical Site Infection

Research studies held contradictory findings about antibiotic preventive measures for reducing SSIs in third molar extractions. Medical evidence points to antibiotics as effective in reducing postoperative infectious complications by a rate of 66% (Risk Ratio 0.34, 95% CI 0.19 to 0.64) which needs 19 patients treated [4]. The data indicates that twenty-two patients will require antibiotic treatment for preventing one infection.

Controlled trials generate inconsistent results because of differences that occur between patient groups as well as surgical methods and infection diagnosis criteria. Directed studies employing clear admission standards and standardized surgical procedures identify reduced overall infection numbers and produce minimal treatment-control group divergence [5,11].

5.2 Effect on Dry Socket

Research about antibiotic prophylaxis as a dry socket prevention method remains uncertain. Research shows antibiotics decrease dry socket occurrence by 34% (risk ratio 0.66, 95% CI 0.45 to 0.97) but treatment for 46 [4] patients results in this outcome.

The administration of antibiotics proved effective in reducing the occurrence of dry socket along with surgical site infections for people who underwent lower third molar extraction procedures [12]. The evidence about antibiotic prophylaxis as a dry socket prevention method is uncertain because studies reveal conflicting results regarding its effectiveness [15].

5.3 Comparative Efficacy of Different Antibiotics

Both amoxicillin (1g) and amoxicillin/clavulanate (875/125mg) were equally effective in preventing infection after third molar extraction, but amoxicillin/clavulanate produced significantly more gastrointestinal discomfort [6]. This highlights the need to balance efficacy with potential adverse effects.Studies of alternative antibiotics for penicillin-allergic patients have shown promising results.

6. Potential Adverse Effects and Concerns

6.1 Antibiotic Resistance

Broad-scale antibiotic prophylaxis causes the most important side effect which is antibiotic resistance development. The inappropriate and excessive prescription of antibiotics leads to resistant bacterial strain formation thus becoming a crucial worldwide health concern [16]. The various microorganisms that exist in the oral cavity function as a resistance gene reservoir [16].

Medical professionals should evaluate the possibility of resistance development against the protective advantages of minimizing non-fatal post-extraction infections in healthy patients with third molars [15]. Society needs to perform detailed risk-benefit evaluations since third molar extractions occur frequently throughout the world.

6.2 Adverse Reactions

The use of antibiotics entails known potential complications considered as mild gastrointestinal side effects through severe allergic reactions. The combination of amoxicillin/clavulanic acid leads to increased gastrointestinal problems than amoxicillin usage alone [6].

People can experience different severity levels of allergic reactions to antibiotics but beta-lactam antibiotics present the most risk for severe anaphylaxis. Routine prophylaxis for third molar extractions poses risk to a small number of patients who are prone to severe allergic reactions (0.01-0.05% for penicillins), a factor that should be considered given the large number of procedures [16].

Patient exposure to antibiotics leads to two main unwanted effects: normal gut microbiota disruption which causes antibiotic-associated diarrhea or Clostridioides difficile infection along with the possibility of serious conditions such as Stevens-Johnson syndrome or toxic epidermal necrolysis occurring rarely [17-20].

6.3 Cost-Benefit Considerations

The financial assessment of antibiotic prophylaxis needs to correlate with medical advantages. Both direct costs arise from antibiotic acquisition but indirect costs include antibiotic side effect management as well as antibiotic resistance development and healthcare resource utilization [11].

Prophylactic antibiotic use for preventing infections or dry sockets after third molar removal demonstrates a demanding number needed to treat 19-46 people to stop a single case thereby making routine prophylaxis less economical for all extractons [4]. The practice of giving antibiotic prophylaxis to specific patients at high risk delivers an optimal balance between medical benefits and financial costs.

7. Recommendations for Clinical Practice

7.1 General Recommendations

Research shows that medical professionals should avoid giving antibiotics as a standard practice for third molar extractions since the majority of simple procedures in healthy patients do not require them [3, 4, 15]. Research shows a low rate of serious infections as an insufficient justification for exposing patients to antibiotic risks [15].

The decision to provide antibiotics before surgical extractions depends on individual procedural requirements [3]. The assessment process needs to base its decision on personal risk evaluation which combines patient-linked features with procedure specific elements [13].

The clinical evidence indicates that healthy patients require a single dose of antibiotics before surgery to achieve optimal prophylaxis [8]. Special high-risk scenarios warrant the use of extended postoperative antibiotic treatments according to the guidelines [3].

7.2 High-Risk Patients

People who have diminished immune function or systemic diseases that impair wound healing or prior surgical site infections usually qualify for antibiotic prophylaxis [3,4]. The healthcare provider should consider antibiotic preventive treatment for patients who have diabetes mellitus, immunosuppressive health conditions, or take immunomodulatory medications [3].The prevention measure is appropriate for patients whose oral structure has specific anatomical risk factors consisting of deeply situated teeth that need extensive bone destruction or teeth with small available distal space [10].

7.3 Alternative Approaches

Additional infection prevention strategies consist of preoperative oral disinfection and precise surgical practices as well as proper wound care and adequate postoperative instructions [1,2].

Multiple studies indicate chlorhexidine mouth rinses provide effective postoperative results by decreasing complications so they function well as an antibiotic substitute method [1]. Evidence about the effectiveness of topical antibiotics used on surgical sites for third molar extractions remains scarce according to studies [2].

8.CONCLUSION

Prevalent use of antibiotics during third molar extractions needs thorough evaluation of patient-specific risk elements together with both positive treatment consequences and adverse drug responses. Research fails to demonstrate that all patients need antibiotic medications before third molar extraction unless they have medical complications or complicated extraction procedures.

Specific patient groups who need antibiotics as prevention can benefit from prophylactic medicine when receiving bone-intensive extractions or when experiencing older age or extended surgical process or weakened immune system. The indication of antibiotic prophylaxis requires just a single preoperative administration of the appropriate medication for prevention purposes.

Given worries about antibiotic resistance, appropriate antibiotic prescriptions in dental practice become essential. The medical professionals need to evaluate the minimal decrease in infection risks versus the extensive dangers posed by antibiotic misuse in public health frameworks. Future investigations should concentrate on developing exact risk assessment methodologies for improving patient selection in prophylaxis and performing targeted prophylaxis evaluations versus standard protocols and exploring antibiotic-independent prevention strategies for surgical complications.

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ORIGINAL RESEARCH

ASSESSMENT OF HEALING OF DENTAL EXTRACTION WOUNDS IN RECOVERED COVID 19 PATIENTS

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ABSTRACT

Background: Wound healing is a physiological process that undergoes series of phases which is well connected. The sequence of the wound healing process is critical and influenced by varied factors that act locally and systemically. The systemic factors that influence the health can affect healing through local factors. Oral cavity is different in many aspects including its scarless healing which happens at a faster rate. SARS CoV2 may influence viability of salivary glands, taste, smell sensations, integrity of oral mucosa, the balance on the microbiota. Recent literature strives need for clarification on post covid effect in oral cavity.

Aim: An Observational Study to Identify influence of SARS Cov2 on Physiological process of Wound Healing after Dental Extraction.

Materials And Methods

Study population: Patients recovering from covid-19 undergoing Tooth extraction. Healthy patients undergoing tooth extraction unaffected by covid-19. 35 cases with history. 35 cases without history of covid were involved. After extraction patients were assessed clinically using Laundry et al criteria. VAS scale used to assess pain intensity.

Results:Covid positive patients on day 3 when assessed with Landry et al healing index for post extraction wound healing, scored 2(33) and 3(2). Bleeding on palpation was noticed on day 3. VAS scored around 1 and 2 for both the patients with, without history. Covid negative patients on day 3 when assessed for post extraction wound healing scored 3(35), bleeding on palpation was not evident on day 3. Covid positive patients on day 7 when assessed for post extraction wound healing, scored 3(16), 4(18), 1(1). Covid negative patients on day 7 when assessed for post extraction wound healing scored good and excellent.

Conclusions: We hypothesize there could be a prolonged early phase of wound healing in dental extraction wounds in patients with history of covid, which could be another feature of long covid syndrome in oral cavity.

Keywords: dental extraction, wound healing, extraction socket and covid 19

INTRODUCTION

Wound healing is a physiological process that undergoes a series of phases and events which is well connected. It involves 4 tightly, precisely arranged phases namely, haemostasis inflammation, proliferation and remodelling. The phases of wound healing are quite structured with the timing, duration and also the intensity. These four phases are found to overlap one another. The sequence of the wound healing process is very critical and can be influenced by varied factors that act locally and systemically. The systemic factors that influence the health as such can have effect on healing directly or indirectly through local factors [1,2]. Covid -19 caused by SARS-CoV-2 is the viral infection that affected about 41,86,50,474 cases in the span of two and half years worldwide(covid19.who.int). The virus causes immune dysfunction and secondary infection leading to respiratory failure. Covid -19 being a respiratory distress virus typically presents with systemic hypoxia and the immune response likely favors capillary dysfunction and also reduces tissue oxygen in the involved tissue [3]. Reduced Tissue hypoxia further induces inflammation which is a doubleedged sword. Oral cavity is a unique environment which stands alone from other tissues in many aspects including its scarless healing which also happens at a faster rate. The fastidious nature could be due to the early onset of inflammatory phase, decreased levels of immune mediators, rapid reepithelization and rapid fibroblast proliferation [4,5]. SARS CoV-2 exhibits neurotropism and mucotropism and may likely influence the viability of salivary glands, taste and smell sensations, integrity of oral mucosa, the balance on the microbiota [6]. Recent literature raising need for clarification on the post covid effect in oral cavity and the surrounding tissues. The possibility of dry socket and the spread of infections to the underlying bone may further impair the healthy healing of the tissue [7-9]. This current study is aimed at assessing the wound healing status of dental extraction in patients recovering from Covid 19. This study was designed and presented in the institutional review board and institutional ethical committee and the study was approved (IEC no: IGIDSIEC2022NRP47UGRKOPM). This study was an ICMR STS project done in the year 2023.

MATERIALS AND METHODS

Study population

Patients recovering from covid-19 undergoing Tooth extraction. Healthy patients undergoing tooth extraction unaffected by covid-19. The patients who visit the Oral and Maxillofacial Surgery department for dental extractions were explained about the study. The patients who were willing to participate in the study, satisfying the exclusion and inclusion criteria were selected and included in the study as study participants. Topical anesthetic gel followed by injection of local anesthetic agent on the specific area to be anesthetized is given for an uneventful extraction. Extraction of the tooth is done using elevator and extraction forceps and once the tooth is pulled out of the socket, the socket should be compressed for minimizing the bleeding, and facilitate clot formation. Post extraction instructions will be given. After the extraction the patients were assessed clinically on the first, third and seventh day [9]. Healing index of Landry, Turnbull and Howley was applied for day 3 and day 7 [10,11]. Pain intensity will be assessed using a 10-degree visual analogue scale (VAS) on all 3 days of observation [12]. Data obtained will be tabulated and inferential statistics would be used for analysis of the data. Chi square test would be used to compare the wound healing status on 3rd and 7th day after extraction among covid and non-covid groups. Detailed proforma with all the details will be filled and documented for each study participant for future References.

Inclusion criteria:

Patients recently recovered from covid-19 undergoing dental extraction, healthy patients undergoing extraction without history of covid.

Exclusion criteria:

Smokers and alcoholics, Diabetics, obese patients, poor nutritional status, patients on medications such as steroids, birth control pills, chemotherapeutic drugs will be excluded since these factors have a role on wound healing.

Sample size calculation:

Sample size calculated using the formula
n = Z21- α /2P(1-P) d2
z (standardization) = 1.96
n = sample size, 35
p (Estimated Proportion) = 9
α (Alpha)=0.05,
d (Estimated error) = 1
N= sample size - 35 cases in study group and 35 cases in control group.

The proportion is derived from the Number of covid positive cases from the covid19dashboard.py.gov.in (127734 reported cases out of 1247953).

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RESULTS

Day 3

Table 1. Comparison of post extraction wound healing status of covid recovering individuals and non-covid healthy individuals

	POOR	GOOD	TOTAL
POSITIVE	33(94%)	2(6%)	35(100%)
NEGATIVE	0(0%)	35(100%)	35(100%)
TOTAL	33(47%)	37(53%)	70(100%)
α^2 =62.43, P<0.001			

Day 7

Table 2. Comparison of post extraction wound healing status in covid recovering and non-covid individuals

	GOOD	VERY GOOD	EXCELLENT	TOTAL
POSITIVE	16(46%)	18(51%)	1(3%)	35(100%)
NEGATIVE	0(0%)	24(69%)	11(31%)	35(100%)
TOTAL	16(23%)	42(60%)	12(17%)	70(100%)
α^2 =25.19, P<0.001				

Day 3

Table 3 Contingency

Covid positive patients on day 3 when assessed with Landry et al healing index for post extraction wound healing, scored 2(33) and 3(2), bleeding on palpation was noticed on day 3. VAS scored around 1 and 2 for both the patients with and without history. Covid negative patients on day 3 when assessed with Landry et al healing index for post extraction wound healing scored 3(35), bleeding on palpation was not evident on day 3.

Contingency Tables				
		DAY 3		
HISTORY OF COVID		2	3	Total
Positive	Count	33.00	2.00	35.00
	% within row	0.94	0.06	1.00
Negative	Count	0.00	35.00	35.00
	% within row	0.00	1.00	1.00
Total	Count	33.00	37.00	70.00
	% within row	0.47	0.53	1.00
CHI SQUARE TEST				
	Value	df	P	
X^2	62.43	1	2.76e - 15	
N	70			

Day 7 Table 4. Contingency

Covid positive patients on day 7 when assessed for Landry et al healing index for post extraction wound healing, scored 3(16), 4(18), 1(1). Covid negative patients on day 7 when assessed with Landry et al healing index for post extraction wound healing scored good and excellent.

Contingency Tables					
		DAY 7			
HISTORY OF COVID		3	4	5	Total
Positive	Count	16.00	18.00	1.00	35.00
	% within row	0.46	0.51	0.03	1.00
Negative	Count	0.00	24.00	11.00	35.00
	% within row	0.00	0.69	0.31	1.00
Total	Count	16.00	42.00	12.00	70.00
	% within row	0.23	0.60	0.17	1.00
CHI SQUARE TEST					
	Value		df		P
X^2	25.19		2		3.39e - 6
N	70				

DISCUSSION

Our association and experiences with covid 19 for the past 2 and half years, made us thoroughly learn about the virus and its mutations and their varied clinical presentations that kept modifying depending on the host, environment, comorbidities and many other factors. Vaccination against SARS CoV-2, has brought down the incidence of newer cases and also the severity of the disease, in cases of new and re-infections. SARS CoV-2 has kept us all in a messed-up state, with its weird post covid manifestations in the affected individuals, which made the term 'Long covid syndrome' come into existence. Transmission of SARS CoV-2 is facilitated by ACE receptors which is also found in oral epithelial cells [13]. The binding of ACE 2 & SARS CoV-2 increases the oxidative stress and endothelial damage. Tissue oxygenation is found to be limited in Covid-19 and reduced oxygenation in turn induce Hypoxia inducible factor and the genes that favor the tissue to adapt the prevailing hypoxia. Optimum Oxygenation promotes wound healing, which is found to be altered in Covid-19 [14]. There is accumulated evidence that covid 19 infected patients, carry at least one symptom for weeks or even months after the acute phase events. Out of 47,910 covid positive patients, 50% reported to have sustained symptoms like fatigue, cognitive dysfunction, shortness of breath. Out of 250000 covid survivors, more than 50% of them had symptoms that was persistent for more than six months [15]. It is utmost important for a clinician to understand these symptoms and also separate these Post covid presentations from the presentations occurring due to other reasons for a better treatment planning and beneficial outcome for the patients. It was observed that 6 in 10 patients with history of covid and hospitalization had post or Long covid syndrome. The reported symptoms were persistent from weeks to more than six months. The common reported symptoms were extreme fatigue, intolerance to walk and any physical activity, loss of stamina, shortness of breath, palpitations, problems in the gastrointestinal tract and modulating weight. Females were presenting more of this long covid syndrome than males [16]. With the evidences of, possibilities of post covid alterations in multiple organs, we decided to study the status of wound healing in dental extraction sockets of patients recovering from covid 19. The duration of the symptoms of covid 19 infections after acute phase is documented to last over 12 months from the time of initial infections. our study included patients recovering from covid, with longest duration one year. all the participants gave history of covid vaccination with 2 doses after the infection. The extraction wounds were observed from day 1 to day 7, the healing index of Landry et al was used to score the healing status. The noncovid control patients gave scores from good to excellent as per the criteria. Granulation tissue was observed on day 3 and no bleeding on palpation was observed, whereas, the group with covid history scored poor and good on day 3, bleeding on palpation was observed on day 3 in participants with covid history. On day 7 the healing was found to improve quiet well in both the groups and scored good, very good and excellent. The bleeding in the extraction sites, observed on day 3 in the group with covid history could be due to the elevated levels of early tissue hypoxia due to the history of covid infection. The improvement in scoring on day 7 in covid positive patients comparing to the day 7 of the covid negative control group could be due to the altered process of wound healing which takes place in an organized fashion. The altered process of wound healing will further delay the wound healing and hence the difference in the two groups. In the current study we hypothesize there could be a possibility of prolonged early phase of wound healing. The study group, presented with symptoms like fever, myalgia, loss of taste, loss of smell, headaches, shortness of breath. Both the groups scored from 1 to 2 in VAS scale and was not a significant finding. However, little significance we observed in our study, results attained from the clinical scoring needs to be confirmed with the levels of hypoxia inducible factor (HIF). Wound healing should be assessed in covid infected individuals with short recovery to remove the confounders. Another major limitation of our study, would be the possibility of the control group to have been infected with covid. Based on the patient's history and records of their tests (PCR, rapid antigen) we selected the patients as covid positive and negative. During the third wave, covid presented with milder flu-like symptoms, which

brought down the need of testing and hospitalization. In addition to this, both the groups were vaccinated and the effect of vaccination on wound healing should be studied. Sample size can be increased to bring down the limitations.

CONCLUSION

It was intended to study the Influence of SARS CoV-2 on the Stepwise Physiological process of Wound Healing after Dental Extraction in individuals recovering from covid and healthy individuals without history of covid. It was observed in the study group that there was bleeding upon palpation in the extraction sites, which could be due the altered early hypoxia which could alter the tissue homeostasis thereby prolonging the early phase of wound healing. The minor differences in scoring on day 7 between the groups could be due to the altered process of wound healing which takes place in an organized fashion. In the current study we hypothesize there could be a prolonged early phase of wound healing in the dental extraction wounds in patients with history of covid, which could be yet another feature of long covid syndrome in the oral cavity. During our study period, we observed few areas where further studies need to be done. The wound healing should be compared with the levels of hypoxia inducible factor (HIF) for its definite causal association. Wound healing should be assessed in covid infected individuals with short recovery to remove the confounders. Another major limitation of our study, would be the possibility of the control group to have been infected with covid. Based on the patient's history and records of their tests (PCR, rapid antigen) we selected the patients as covid positive and negative. During the third wave, covid presented with milder flu like symptoms, which brought down the need of testing and hospitalization. In addition to this, both the groups were vaccinated and the effect of vaccination on wound healing should be studied. The effect of long covid syndrome in oral cavity and its associated structures should be studied for any long-term changes at cellular and tissue level.

DECLARATIONS

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This study was done as ICMR – STS project in 2023 and was approved by ICMR and was satisfactory.

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As it was an ICMR – STS project, the funds were provided by ICMR.

Ethical Approval

This study was designed and presented in the institutional review board and institutional ethical committee and the study was approved (IEC no: IGIDSIEC2022NRP47UGRKOPM).

Informed Consent

Informed Consent was obtained from all the patients who were willing to participate in the study, and were explained about the procedure.

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ORIGINAL ARTICLE

EVALUATION OF THE PREVALENCE AND MORPHOMETRIC CHARACTERISTICS OF THE LATERAL LINGUAL CANAL IN THE INTER-FORAMINAL MANDIBLE OF THE MELMARUVATHUR POPULATION: A RETROSPECTIVE CBCT STUDY

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ABSTRACT

Objectives: Lateral lingual canal (LLC) is a critical anatomical landmark for presurgical assessment of dental implants. Data on the prevalence and morphometry of the LLC in varying population shows a lot of variability. The purpose of this cross-sectional Cone Beam Computed Tomography (CBCT) study was to assess the frequency, distribution, location , and morphometric variations of the LLC of Melmaruvathur population in South India.

Materials and Methods:A retrospective cross-sectional study was conducted using 200 CBCT scans of partially edentulous individuals aged 21–72 years from the Department of Radiology at Adhiparasakthi Dental College and Hospital. Inter-foraminal region of mandibular scans were assessed for the presence, location, course, and morphometric parameters of the LLC. Statistical analysis was performed using Chi-square and Mann-Whitney tests with a significance threshold of $p<0.05$.

Results: LLCs were observed in 22% of participants, with 20% showing a single canal and 2% exhibiting double canals. The most common location was the canine region (50%), followed by the premolar (41.66%) and lateral incisor (8.33%) region, with a significant gender-based association ($p=0.048$). Cephalic course was the most frequent (66.66%), and 31.25% of canals crossed a critical diameter of 1 mm. No significant gender differences were observed in morphometric measurements.

Conclusion: LLCs exhibit notable anatomical variability in the Melmaruvathur population, particularly in their prevalence, location, and course. Given their potential to cause intraoperative complications, especially during implant surgery, routine and thorough preoperative CBCT evaluation is recommended to ensure surgical safety and precision in implant surgery.

Keywords**:** Lateral Lingual Canal, Inter-foraminal mandible, Cone-beam Computed Tomography (CBCT), Morphometric variability, Mandibular Anatomy

INTRODUCTION

The human mandible is a highly complex and varied anatomical structure with significant clinical implications, particularly in fields of implantology, oral surgery, and radiology. A key landmark in this region is the Median Lingual Canal and Lateral Lingual Canal (LLC), which harbor anastomosing blood vessels . Lingual surface of mandible shows a high frequency of foramina which can be classified as median lingual foramina and lateral lingual foramina[1]. Vascular canal emerging from lingual foramina in the midline in and around genial tubercles is known as Median Lingual Canal or median Vascular Canal which have shown a prevalence of 90-100%. Accessory Vascular canals originating from the lingual foramina on the lingual mandibular surface other than the midline are called as LLC. The LLC is frequently identified during routine CBCT scans, but its clinical significance is often underestimated[2]. Misinterpretations or failure to recognize variations in LLC morphology can result in unintended complications during procedures such as implant placement, bone harvesting, and surgical extraction[3].

Numerous case reports have documented life-threatening complications due to profuse bleeding, edema, and hematoma formation during or immediately after surgical procedures in the inter-foraminal region[2,3,4]. Understanding the typical and atypical features of the LLC is vital for clinicians to reduce such risks.

Studies on the morphology of the lateral lingual canal have shown significant variability in terms of its prevalence, distribution, and morphometric characteristics[5]. Moreover, while studies have explored the LLC in various populations restricting to urban population, limited research has been conducted on specific regional populations like Melmaruvathur. The Melmaruvathur population presents unique anthropological and genetic characteristics that could potentially result in distinct morphometric features compared to other populations. This study addresses the gap by providing population-specific data, thus enriching the literature.

In context to this subpopulation, a previous study evaluated the morphological variability of the median lingual canal using Cone Beam Computed Tomography (CBCT) shedding light on the anatomical peculiarities specific to this region[6]. Extending this knowledge to the LLC offers a more comprehensive understanding of the variability in lingual canal anatomy within the same population.

The primary objective of this study was to evaluate the prevalence, distribution, and morphometric variability of the lateral lingual canal in the inter-foraminal region of mandible in the Melmaruvathur population, using CBCT imaging. By providing detailed insights into the anatomical variations of this vascular structure, the study aims to enhance the safety and precision during surgical interventions in the inter-foraminal region in this Indian Subpopulation.

MATERIALS AND METHODS

Study Design

A retrospective cross-sectional study was conducted to evaluate the frequency, localisation, course and morphometric variation of LLC in the inter-foraminal mandible among individuals from the Melmaruvathur region in South India using cone-beam computed tomography (CBCT). The study protocol received ethical approval from the Institutional Review Board of Adhiparasakthi Dental College and Hospital, Melmaruvathur (Approval No: IRB/27/ Prostho-02/APDCH/2025). Written informed consent was taken from all the participants before surgery in accordance with NABH guidelines. All procedures were performed in accordance with the ethical principles of the Declaration of Helsinki.

Participant Selection and Imaging Protocol

CBCT scans from 200 participants were retrospectively selected from the archives of the Department of Radiology, APDCH. Based on the lateral lingual foramen prevalence of 13.3%[7], the sample size calculated was 178 using OpenEpi, version 3 (www.OpenEpi.com). No scan was performed exclusively for this study. Participants included were partially edentulous adults aged 21–72 years with at least one missing mandibular tooth for whom CBCT had been taken for planning implant surgery from the period of January 2023 to December 2024. Patients having taken CBCT for wisdom tooth extraction were also included in the study. All CBCTs were of medium FOV. Exclusion criteria included a history of craniofacial trauma, jaw surgery, congenital anomalies, orthodontic treatment, systemic diseases like osteoporosis, edentulous mandibular arch, syndromic conditions, unclear scans, and small or large FOV CBCT . All CBCTs were obtained using a Dentsply Sirona CBCT unit operating at 85 kVp and 6 mA, with a medium field of view (8 × 8 cm) and 1 mm slice thickness.

Image Analysis

CBCT scans were independently reviewed by two implantologists with over 5 years of experience, using CareStream 3D Imaging Software (Version 3.10.38; Carestream Dental LLC, Atlanta, USA). Observers adjusted brightness and contrast to enhance visualization. All measurements were recorded to a single decimal point. In cases of interobserver disagreement, an oral radiologist re-evaluated the scan, and their interpretation was taken as final.

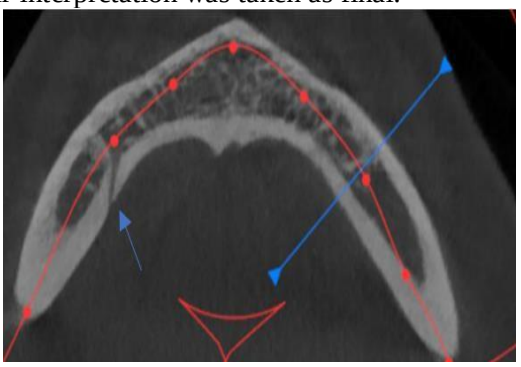


Figure.1 Axial view depicting lateral lingual canal (arrow)

The mental foramen was used as a starting reference point, and scans were cropped from mental Foramen on either side. The LLC was identified by distinct cortication and analyzed in both axial and cross-sectional views (Fig 1). Canal seen in the midline in genial tubercle region were named as MLC and ones identified away from midline of mandible were named as LLC as classified by Sanomiya *et.al* [8]. The interobserver agreement for the identification and classification of the mandibular lingual canal was substantial, with a Cohen's kappa (κ) value of ≥ 0.70,

reflecting a high level of consistency between the evaluators. The LLC was assessed for number and location relative to the tooth region(Lateral Incisor, Canine and Premolar) and its supero-inferior course was categorized as caudal, transverse, or cephalic in cross section. Course Localisation of canals were done by drawing a vertical line across the centre of the tooth. The LLC foramina nearest to the central line determined the location of the canal designated as Lateral Incisor region, Canine region and Premolar region. Morphometric parameters measured included:

- LLC diameter at emergence on the lingual mandibular plate
- LLC diameter at exit(intraosseous)
- Distance from LLC emergence to buccal cortical plate
- Distance from LLC exit to buccal cortical plate
- Distance from LLC emergence to the inferior border of the mandible (IBM)
- Distance from LLC exit to the IBM

All measurements were taken from standardized cross-sectional slices.

RESULTS

Analysis of 200 CBCT scans was done with a Male to Female ratio of 1:1. Study included 100 males and 100 females with mean age of 45.3 ± 13.1 years. Mean age of females and males was (42.8 ± 14.9) years and (47.8 ± 13.2) years respectively. LLC was seen in 44(22%) patients and 156(78%) patients had no LLC. Prevalence of LLC and no association with gender is shown in **Table1**. 40(20%) patients showed single accessory canal and 4(2%) patients had double accessory canal in interforaminal region . Total Number LLC present were 48.(**Table2**). Total Number LLC present were 48.

Table 1. Presence of LLC

Gender	LLC		p-value
	Absent n (%)	Present n (%)	
Male	80 (40)	20 (10)	0.495 (NS)
Female	76 (38)	24 (12)	
Total	156 (78)	44 (22)	

NS - Statistically not significant at p<0.05, Chi-square test

Table2. Number of LLC

Gender	LLC			p-value
	0 n (%)	1 n (%)	2 n (%)	
Male	80 (40)	20 (10)	0 (0)	0.129 (NS)
Female	76 (38)	20 (10)	4 (2)	
Total	156 (78)	40 (20)	4 (2)	

NS - Statistically not significant at p<0.05, Chi-square test

24 (50%) accessory canals were present in canine region, 4(8.33%) were present in lateral incisor region and 20(41.66%) were present in premolar region(**Table 3**).

Table 3. Association between location of LLC and Gender

	Canine n(%)	Lateral incisor n(%)	Premolar n(%)	p-value
Male	8 (40)	4 (20)	8 (40)	0.048*
Female	16 (57.14)	0 (0)	12 (42.85)	
Total	24 (50)	4 (8.33)	20 (41.66)	

*Statistically significant at p<0.05, Chi-square test

32(66.66%) canals were seen on right side and 16(33.33%) on left side(**Table 4**).

Table4.Association between Regional Distribution of LLC and Gender

	Left n(%)	Right n(%)	p-value
Male	4 (33.33)	8 (73.33)	0.110 (NS)
Female	12 (60)	8 (40)	
Total	16 (33.33)	32 (66.66)	

NS- Statistically not significant at p<0.05, Chi-square test

Table 5. Association between course of LLC and Gender

	Caudal n(%)	Cephalic n(%)	Transverse n(%)	p-value
Male	8 (40)	12 (60)	0 (0)	0.051 (NS)
Female	4 (14.28)	20 (71.42)	4 (14.28)	
Total	12 (25)	32 (66.66)	4 (8.33)	

NS- Statistically not significant at p<0.05, Chi-square test

32(66.66%) canals showed a cephalic course, 4(8.33%) showed a transverse course and 12 (25%) showed a caudal course. Association between the course of LLC and gender is seen in **Table 5**. 15(31.25%) canals showed a diameter greater than 1mm with no statistically significant association with gender. Mean of all the morphometric measurements of LLC and its association with gender is seen in the **Table 6**.

Table 6. Linear measurements of LLC and association with gender

		Total	Gender		p-value
			Male	Female	
Diameter at entrance of LLC	Mean±SD	0.655±0.254	0.334±0.164	0.857±0.344	0.230 (NS)
Diameter at exit of LLC	Mean±SD	0.422±0.224	0.419±0.208	0.426±0.240	0.321 (NS)
Distance from entry of LLC to inferior border of mandible	Mean±SD	4.036±2.088	4.707±2.220	3.249±1.956	0.481 (NS)
Distance from exit of LLC to inferior border of mandible	Mean±SD	4.489±2.410	4.486±2.176	4.504±2.644	0.334 (NS)
Distance of canal entry from buccal plate	Mean±SD	4.149±2.278	4.009±1.948	4.279±2.6080	0.210 (NS)
Distance of canal exit from buccal plate	Mean±SD	2.931±1.518	2.772±1.344	3.085±1.692	0.373 (NS)

NS- Statistically not significant at p<0.05, Mann Whitney test

DISCUSSION

This retrospective CBCT-based study aimed to assess the prevalence, distribution, trajectory, and morphometric characteristics of the lateral lingual canal in the inter-foraminal region of the mandible in a South Indian subpopulation. The findings offer valuable insights for clinicians performing implant placement or other surgical procedures in this anatomically critical area. In recent years, advancements in imaging technologies, particularly CBCT, have provided more precise and detailed three-dimensional views of mandibular structures. CBCT offers a clear advantage over traditional radiographic methods, providing high-

resolution images at a reduced radiation dose, making it an ideal modality for the detailed study of the lateral lingual canal[9]

The LLC was identified in 22% of the study population, which is in line with previous studies reporting prevalence rates ranging from 11.5% to 39%[7,8,5]. The variability in prevalence may be attributed to ethnic differences, imaging resolution, and methodological variations. Gender-wise distribution of the LLC showed no statistically significant difference, although double LLC were observed exclusively in females (2%), which may suggest an observation requiring further investigation. Most LLCs were unilateral and singular, with only 2% of participants exhibiting double canals. In contrast to the findings of our study, a study on Iranian population showed that LLC was 1.36 times more common in males than females[7] .

The anatomical region most frequently associated with the LLC was the canine region (50%), followed by the premolar (41.66%) and lateral incisor (8.33%) regions in our study. A statistically significant association was observed between gender and regional distribution ($p = 0.048$), with females showing more frequent LLCs in the canine region. These results highlight the need for careful radiographic evaluation of the canine region, particularly in females in the population of Melmaruvathur in South India . A cadaveric study conducted in South India also reported the canine region as the most common region for the presence of LLC[10] . In contrast to our study other studies targeting different ethnicity have shown premolar region as the commonest site for the presence of LLC[1,5,7] .One probable reason for this variation could be the area scrutinised for the presence of LLC in various studies. Our study targeted only the inter-foraminal region, whereas the study done on Iranian population assessed the presence of LLC from second molar on either side[7]. Sahman *et.al* have targeted 2nd Premolar on either side to assess the presence of accessory foramen[5]. This variation in methodology could have also led to differences in the prevalence of LLC in the studies. This finding underscores the importance of careful planning and technique when placing implants in this region to minimize the risk of hemorrhagic events. In consensus to our study a systematic review identified that the mandibular canine region is the area most susceptible to bleeding complications during dental implant procedures[11] **.** A similar CBCT study assessing accessory canals in mandible of 50 Indian subjects showed the presence of 89% canals in the interforaminal region and 11% canals present in posterior to mental foramen[12]**.**

Regarding the canal trajectory, a cephalic course was most commonly observed (66.66%), followed by caudal (25%) and transverse (8.33%) orientations. These findings are comparable to those reported in the study by Kawai et al[13]; who noted a predominance of cephalic trajectories. However; Liang et.al[14] found 72% of the canals in his study had courses running downwards. Clinically, cephalic and transverse orientations may pose a higher risk of neurovascular injury during implant osteotomy. 31.25% LLC demonstrated a diameter of ≥1 mm in our study. Critical diameter of 1mm is considered clinically relevant due to higher risk for bleeding during osteotomy[15]. A similar study done in South India showed that 61% of the lingual vascular canals had a diameter of greater than 1mm[16]. A wide difference in the percent could be attributed to the methodology and regional difference in the population. While the mean diameters and linear measurements did not differ significantly between males and females, the wide range in values observed underscores the high anatomical variability of these canals. This reinforces the necessity for individualized radiological assessment rather than reliance on generalized anatomical assumptions.

The morphometric parameters including distances from the buccal cortical plate and the inferior border of the mandible showed no significant gender-based differences, though they revealed a broad distribution, further emphasizing the variability in the course and emergence of the LLC.

Knowledge of the prevalence, location, and trajectory of the LLC is essential for minimizing complications during surgical procedures in the anterior mandible. Injury to the LLC can result in persistent bleeding, hematoma formation, or neurosensory disturbances. The findings support the routine use of CBCT imaging for accurate preoperative planning and risk mitigation. This study was limited by its retrospective design and reliance on medium field-of-view CBCT scans, which, although sufficient, may not have captured finer canal details compared to micro-CT. Furthermore, the sample was derived from a single geographic region, potentially limiting the generalizability of results. Prospective studies with larger and more diverse populations are recommended to validate these findings.Canal course studied was only in cross sectional view. Course in the axial view was not studied. Parametric association with the edentulous state of the patient and age was not done in this study.

CONCLUSION

This CBCT-based study demonstrated that the lateral lingual canal is present in 22% of individuals in the Melmaruvathur population, most frequently in the canine region and often with a cephalic trajectory. Although the LLC showed no significant gender-based morphometric differences, its variable presence and dimensions highlight the importance of individualized preoperative assessment. The findings underscore the need for careful planning during surgical procedures in

the inter-foraminal region to avoid neurovascular complications. Routine use of CBCT imaging is strongly recommended to enhance surgical safety in implantology and other mandibular interventions.

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

MORPHOMETRIC ANALYSIS OF THE MENTAL FORAMEN IN SOUTH INDIAN POPULATION

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Abstract

Introduction: The mental foramen, a critical anatomical landmark on the mandible, transmits the mental nerve and vessels, and its precise location, shape, and size are vital for dental surgical procedures, anesthesia, and forensic applications. Variations in its morphology and the presence of accessory mental foramina can impact clinical outcomes.

Aim & Objectives: This study aimed to evaluate the morphologic and morphometric characteristics of the mental foramen and the incidence of accessory mental foramina in dry human mandibles using stainless steel Vernier calipers.

Materials and Methods: A total of 150 adult dry human mandibles from a South Indian population were examined. Measurements included the shape, size, and position of the mental foramen relative to the symphysis menti, posterior border of ramus of the mandible, alveolar margin, and inferior border of body of the mandible. The presence of accessory mental foramina was also recorded. Statistical analysis was performed to assess bilateral differences using t-tests ($p<0.05$).

Results: The mental foramen was predominantly oval (77.3%) and located in line with the longitudinal axis of the second premolar (type 4, 58.6% on right; 57.3% on left). Mean distances from the symphysis menti to the anterior margin of the mental foramen were 25.12 ± 2.31 mm on the right side and 25.02 ± 2.24 mm on the left side; p=0.359). The mean distance from the posterior margin of the mental foramen to the posterior border of the mandible was 62.54 ± 6.18 mm on the right side and 63.06 ± 5.23 mm on the left side; p=0.213). Vertical distances from the alveolar margin to the upper margin of the mental foramen were 10.81 ± 2.81 mm on the right side and 10.74 ± 2.81 mm on the left side; p=0.407), and from the lower margin to the base of the mandible were 12.48 ± 1.91 mm on the right side and 12.50 ± 1.91 mm on the left side; p=0.451). Accessory mental foramina were observed in 10.56% of mandibles, typically below the first molar.

Conclusion: The study confirms the predominant oval shape and second premolar alignment of the mental foramen, with no significant bilateral differences in morphometric measurements. The incidence of accessory mental foramina underscores the need for careful preoperative assessment. These findings enhance the anatomical knowledge base for dental surgeons, aiding in safer surgical planning and anesthesia administration.

***Keywords*:** Mental Foramen, Accessory Mental Foramen, Mandible, Morphometry, Dental Surgery

INTRODUCTION

The complex array of foramina pervading the craniofacial skeleton is essential passageways for neurovascular structures, and they are responsible for sensory and motor innervation and vascular supply to the

face and cranium[1].

Foramina such as those of the trigeminal nerve are responsible for transmitting nerves and vessels, and locations and diameters are utmost important for surgical and diagnostic interventions[2]. The mental foramen, a very critical anatomical landmark on the anterior surface of the mandible, is of critical clinical significance for dental and surgical treatment. Accurate information about the position and morphometry of the mental foramen is essential for some dental treatments, including dental implant insertion, periodontal surgery, and endodontic procedures, and surgical interventions on the mandible[3].

The mental foramen is an opening resembling a funnel on the anterolateral aspect of the mandibular body, about 11-15 mm above the inferior border. It allows passage for the mental nerve and vessels. The mental nerve is an extension of the inferior alveolar nerve, supplying sensory innervation to the lower lip, vestibule, and gingival mucosa up to the first premolar tooth. The mean diameter of the foramen is 4.6 mm in the horizontal plane and 3.4 mm in the vertical plane. Most mental foramina are posterosuperiorly oriented, either along the longitudinal axis of the second premolar tooth or between the first and second premolars. The orientation of the mental foramen also alters with age - it is anteriorly oriented prior to mandibular fusion during the first few years of life, afterwards becoming posterior following the second or third year. The position of the mental foramen differs between racial groups. In Mongoloid groups, it aligns with the second premolar, whereas in Black groups, it is behind the second premolar. In Caucasians, the mental foramen is in front of the second premolar[4]. The site of the mental foramen depends mostly on age, sex, race, and dental status.

The occurrence of nerve fibers within accessory foramina could be of importance in the efficacy of local anesthesia after a standard inferior alveolar nerve block. Also, blood vessels located in accessory foramina can be a source of intra osseous hemorrhages in implant procedures[5]. Notably, the mental foramen is usually challenging to identify since it is not clinically visible and palpable. Hence, understanding of the mental foramen is necessary to allow diagnostic, surgical, local anesthetic and other invasive oral and maxillofacial procedures, and to avoid injury during surgical and anesthetic techniques which could lead to paresthesia and total loss of sensation. And the research on the mental foramen is necessary not only for surgical and diagnostic purposes, but also for identifying possible variations in various populations.

2. MATERIALS AND METHODS

Our study includes 150 dry human mandibles of unknown sex and age collected from department of Anatomy of Osmania medical college, Hyderabad and various other medical colleges in and around Hyderabad. Random selection of mandibles was done, labeled and measurements were taken. Data collected entered in Microsoft excel sheets, tables and graphs are generated by statistical software XLSAT2017. 'p' and 't' values are generated by using Student's 't' test by SPSS 12.0 statistical software.

The following parameters of mental foramen of each mandible are taken for the study.

A. Non-Metric Parameters.

1. The shape of mental foramen.
2. The position of mental foramen.
3. Presence of accessory mental foramen. (These were recorded by naked eye examination.)

B. Metric Parameters.

1. Size of mental foramen. (Measured with the help of vernier calipers.)
   a. Horizontal diameter of mental foramen.
   b. Vertical diameter of mental foramen.
2. Location of mental foramen in relation to mandibular parameters. (Measured with the help of vernier calipers.)
   a. Distance from symphysis menti to anterior margin of mental foramen.
   b. Distance from posterior border of ramus of mandible to posterior margin of mental foramen.
   c. Distance from alveolar margin to upper margin of mental foramen.
   d. Distance from the lower border of the body to the lower margin of mental foramen.

3.RESULTS

A. Non-Metric Parameters:

3.1. The Shape of Mental Foramen

In our study150 dry human mandibles, shape of mental foramen is observed by naked eye examination. The various shapes found in this study are oval shape, round shape. Oval shape is the most common shape, and round is the next common shape in this study.

On the right side the oval shape is seen in 116 mandibles (77.3%), on the left side oval shape is seen in 119 mandibles (79.3%). On the right side round shape is seen in 34 mandibles (22.6%), on the left side round shape is seen in 31 mandibles (20.6%).

Table 1. The Shape of Mental Foramen

S.NO	SHAPE	RIGHT SIDE	LEFT SIDE
1	Oval	116(77.3%)	119(79.3%)
2	Round	34(22.6%)	31(20.6%)
Total		150(100%)	150(100%)

3.2. Position of Mental Foramen:

In present study position of mental foemen is observed by naked eye examination.

Positioning relations to the roots of mandibular teeth may vary. According to Tebo and Telford positions divided as 6 types (Balakrishnan et al., 2018; Sevmez, Bahşi and Orhan, 2021).

1. between the canine and the first premolar.
2. beneath the first premolar
3. between the premolars
4. beneath the second premolar
5. between the second premolar and first molar
6. beneath the root of the first molar

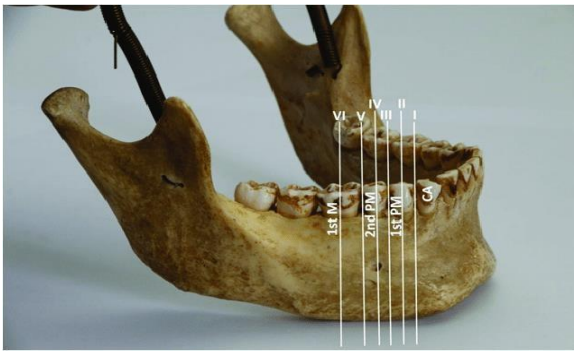


Figure 1. Diagram Showing Position of Mental Foramen in Relation to Mandibular Teeth

Most common position on the right side is type 4 seen in 88 mandibles (58.6%), followed by type 3 seen in 28 mandibles (18.6%), type 5 is seen in 20 mandibles (13.3%), type 6 is seen in 8 mandibles (5.33%). Most common position on the left side is type 4 seen in 86 mandibles (57.3%), followed by type 3 seen in 34 mandibles (22.6%), type 5 is seen in 19 mandibles (12.6%), type 6 is seen in 5 mandibles (3.33%). 6 mandibles are edentulous. Hence, unable to locate the position of mental foramen.

Table 2. Percentage Distribution of Position of Mental Foramen

S.No	Position	Right	Left
1	Type1	-	-
2	Type2	-	-
3	Type3	28(18.6%)	34(22.6%)
4	Type4	88(58.6%)	86(57.3%)
5	Type5	20(13.3%)	19(12.6%)
6	Type6	8(5.33%)	5(3.33%)

3.3. Presence of Accessory Mental Foramen

In our study the presence of accessory mental foramen is observed by naked eye examination. Accessory mental foramen is found on the right side in 9 mandibles (6%) and on left side in 2 mandibles (1.33%).

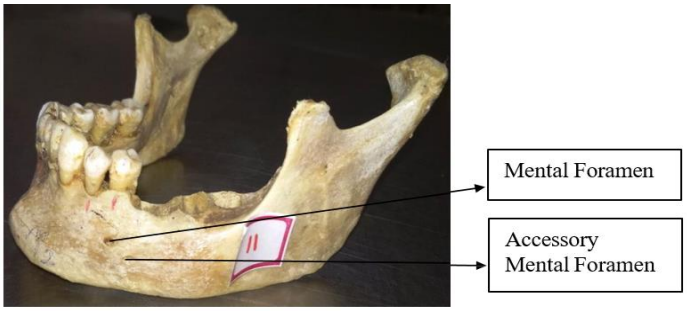


Figure 2. Diagram Showing Presence of Accessory Mental Foramen

S.NO	SIDE	RIGHT	LEFT
1	Number of accessory mental foramen	9(6%)	2(1.33%)

3.4. Size of Mental Foramen

The size of mental foramen is measured by taking horizontal diameter and vertical diameter of mental foramen with help of digital Vernier caliper.

a. Horizontal diameter of mental foramen

In present study horizontal diameter of mental foramen on right side ranges between 1.03-5.12 mm with arithmetic mean of 2.6779 mm with median 2. 65 mm and standard deviation is found to be 0.85681mm. On the left side ranges between 1.01- 4.24 mm with an arithmetic mean of 2.63597 mm with a median of 2.47 mm and standard deviation is found to be 0.8356 mm. t-value is 1.65001, p-value is 0.3345. Statistically there is no significance is seen.

Table 4. Incidence of Horizontal Diameter of Mental Foramen

S.No		Right	Left
1	Minimum	1.03mm	1.01mm
2	Maximum	5.12mm	4.24mm
3	Mean	2.67792mm	2.63597mm
4	Median	2.65mm	2.47mm
5	Standard Deviation	0.85681mm	0.8356mm
6	t-value	1.65001	
7	p-value	0.3345	

b.Vertical diameter of mental foramen: In present study vertical diameter of mental foramen on right side ranges between 1.05-4.67 mm with arithmetic mean 2.05309 mm with median 1.96 mm. Standard deviation found to be 0.63741 mm. On left side ranges between 1.01-4.13 mm with arithmetic mean 2.14866 mm with median 2.04 mm. Standard deviation found to be 0.68416 mm. t-value is 1.65001, p-value is 0.1065. Statistically there is no significance that is present.

Table 5. Incidence Of Vertical Diameter of Mental Foramen

S.NO		RIGHT	LEFT
1	Minimum	1.05mm	1.01mm
2	Maximum	4.67mm	4.13mm
3	Mean	2.05309mm	2.14866mm
4	Median	1.96mm	2.04mm
5	Standard deviation	0.63741mm	0.68416mm
6	t-value	1.65001	
7	p-value	0.1065	

3.5. Location of Mental Foramen with Respect to Mandibular Parameters:

In our study the position of mental foramen with respect to the borders were also measured with help of digital Vernier calipers.

a. **Distance from symphysis menti to anterior margin of mental foramen:**

The distance between symphysis menti to anterior margin of mental foramen ranges between 17.02-30.66 mm on right side with arithmetic mean 25.1187 mm with 25.15 mm median and standard deviation 2.31048 mm. On left side ranges between 18.01-30.56 mm with arithmetic mean of 25.0241 mm median 25.06 mm. Standard deviation 2.23835 mm. t-value is 1.65001, p-value is 0.3598. Statistically there is no difference between right and left sides of this parameter.

Table 6. Incidence Of Distance Between Symphysis Menti to Anterior Margin of Mental Foramen

S.No		Right	Left
1	Minimum	17.02mm	18.01mm
2	Maximum	30.66mm	30.56mm
3	Mean	25.1187mm	25.0241mm
4	Median	25.15mm	25.06mm
5	Standard deviation	2.31048mm	2.23835mm
6	t-value	1.65001	
7	p-value	0.3598	

b. Distance from posterior border of ramus of mandible to posterior margin of mental foramen.

The distance from the posterior margin of mental foramen to posterior border of mandible ranges between 31.88-70.98 mm on right side with an arithmetic mean of 62.537 mm, median 63. 01 mm and standard deviation is 6.18123 mm. On the left side ranges between 31.82-71.47mm with the arithmetic mean of 63.0644mm, median of 63.31 mm and standard deviation of 5. 2323 mm. t-value is 1.65016, p-value is 0. 2136. statistically there is no significance seen.

Table 7. Incidence Of Distance from Posterior Margin of Mental Foramen to Posterior Border of Ramus Mandible.

S.No		Right	Left
1	Minimum	31.88 mm	31.82 mm
2	Maximum	70.98 mm	71.47 mm
3	Mean	62.537 mm	63.06 mm
4	Median	63.01 mm	63.31 mm
5	Standard Deviation	6.18123 mm	5.23 mm
6	t-value	1.65016	
7	p-value	0.2136	

b. Distance from alveolar margin to upper margin of mental foramen

The distance from alveolar margin to upper margin of mental foramen ranges between 3.19-16.34mm on right side with mean 10.814 mm, median 11.33mm and standard deviation 2.80834 mm. On left side ranges between 3.66-18.88mm with mean 10.7376mm, median 11. 18mm and standard deviation 2. 81248 mm. t-value is1.65001, p-values 0. 4073. Statistically there is no significance present.

Table 8. Incidence Of Distance from Alveolar Margin to Upper Margin of Mental Foramen of Mandible

S.No		Right	Left
1	Minimum	3.19 mm	3.66 mm
2	Maximum	16.34 mm	18.88 mm
3	Mean	10.814 mm	10.7376 mm
4	Median	11.33 mm	11.18 mm
5	Standard Deviation	2.80834 mm	2.81248 mm
6	t-value	1.65001	
7	p-value	0.4073	

c. Distance from the lower border of the body to the lower margin of mental foramen: The distance from lower margin of mental foramen to base of mandible ranges between 6.63-16.98 mm on right side with mean 12.4752 mm, median 12.56 mm. Standard deviation 1.90751 mm. On left side, ranges present between 6.35-16.98 mm with mean 12.5022 mm, median 12.65 mm. Standard deviation 1.91399 mm. t-value is1.65001, p-value is 0.4515. Statistically there is no significance that is present.

Table 9. Incidence of Distance from Lower Margin of Mental Foramen to Base of Mandible

S.No		Right	Left
1	Minimum	6.63mm	6.35mm
2	Maximum	16.98mm	16.98mm
3	Mean	12.4752mm	12.5022mm
4	Median	12.56mm	12.65mm
5	Standard Deviation	1.90751mm	1.91399mm
6	t-value	1.65001	
7	p-value	0.4515	

DISCISSION

A. Non-Metric Parameters:

4.1. Shape of the Mental Foramen:

According to standard textbooks, mental foramen shape is an oval shaped opening located at anterolateral aspect of the body of the mandible. According to the data assessed in the study Most common shape is oval shape seen on right side in 116 mandibles (77.3%), on left side is seen in 119 mandibles (79.3%). And the next common shape is round seen on the right side in 34 mandibles (22 .3%), on left side round shape is seen in 31 mandibles (20.6%).

The findings of the present study are consistent with these prior observations. Specifically, several studies have found the oval shape to be predominant, accounting for 83.3% to 88.6% of cases bilaterally [6-8]. The round shape was less common, reported in 11.4% to 16.7% of cases[6,8]. These results from the literature align with the findings of the current investigation. Variations in the reported prevalence of oval and round shapes across studies may be attributed to differences in sample size and population characteristics. Overall, the available evidence suggests that the oval shape is the most frequently encountered morphology of the mental foramen. The importance of shape of mental foramen is, it is widely variable in different individuals, helpful in racial identification.

4.2. Position of the Mental Foramen:

Position in relation to the roots of mandibular teeth may vary. According to Tebo and Telford positions are divided into 6 types. Type1- between the canine and the first premolar, type 2- beneath the first premolar, type 3 - between the premolars, type 4- beneath the second premolar, type 5- between the second premolar and first molar, and type 6 - beneath the root of the first molar.

In our study the most common position of mental foramen in relation to the mandibular teeth is type 4 seen in 88 mandibles (58.6%) on right side, on left side 86 mandibles (57.3%). Type 3 is seen in 28 mandibles (18.6%) on the right side, 34 on the left side (22.6%), type 5 seen in 20 mandibles (13.3%) on the right side,19 mandibles (12.6%) on left side. Type6 is seen in 8 mandibles (5.33%) on the right side, on the left side 5 mandibles (3.33%). The positional distribution of mental foramen, particularly the prevalence of type 4 and the least common positional types, has been extensively studied, with findings revealing both consistencies and variations when compared to the present study. Oliveira Junior et al. (2009)[9] reported a bilateral prevalence of type 4 at 45.17%, which is notably lower than the prevalence observed in the present study. Additionally, their identification of type 6 as the least common position, with 3.22% on the right side and 1.52% on the left side, similarly reflects lower values compared to the current findings. These differences may suggest variations in sample populations or methodological approaches, such as imaging techniques or classification criteria. In contrast, Devi K Sankar et al. (2011)[10] reported a higher bilateral prevalence of type 4 at 73.2%, slightly exceeding the present study's findings. However, their observation of type 5 as the least common position (7.8% bilaterally) diverges from the current study, indicating potential discrepancies in anatomical distribution or diagnostic criteria across study populations.

Rakhi Rastogi et al. (2012)[11] and Virendra Budhiraja et al. (2012)[7] both corroborate the present study's identification of type 4 as the most common position, with prevalences of 52.8% on the right side and 54% on the left side in the former, and 61% on the right side and 59% on the left side in the latter. However, their findings on the least common types differ, with Rastogi et al. identifying type 2 (3.4% bilaterally) and Budhiraja et al.[7] identifying type 6 (0.9% right, 1.9% left). Ajay Parmar et al. (2013)[12] and K Udhaya et al. (2013)[13] further support the predominance of type 4, with prevalences of 64.7% on the right side and 66.7% on the left side in the former, and 52.22% on the right side and 51.11% on the left side in the latter, aligning closely with the present study. However, while Parmar et al.'s identification of type 6 as the least common (3.9% right, 2% left) is consistent with the current findings, Udhaya et al.'s report of type 2 (4.44% bilaterally) as the least common does not align, underscoring variability in less prevalent positional types. Rahul Rai et al[14]. (2014) reported a notably higher bilateral prevalence of type 4 at 81.52%, surpassing the present study's findings. Their identification of type 6 as the least common (7.9%) aligns with the current study, suggesting consistency in the lower prevalence of this type. Similarly, Kasat PA et al. (2016)[16] and Muna Kadel et al.[17] (2016) reported type 4 as the most common position (46.5% bilaterally and 58% right/69% left, respectively) and type 6 as the least common (2% bilaterally and 2% right/3% left, respectively), both of which are consistent with the present study's findings.

The consistent identification of type 4 as the most prevalent position across all reviewed studies underscores its anatomical significance.

However, the variability in prevalence rates and the least common positional types suggests potential influences from factors such as sample size, population demographics, or methodological differences in classification or imaging techniques. Additionally, population-specific anatomical variations, possibly influenced by genetic or environmental factors, could account for the observed differences. Clinical importance of position of mental foramen: mental foramen is difficult to localize as there are no absolute anatomical landmarks for reference. The mental foramen cannot be visualized or palpated clinically; hence it is localized in relation to the lower teeth. So, knowledge of the most common position of the mental foramen in relation to lower teeth is important for dental surgeons both while administering regional anesthesia and performing periapical surgeries of mandible.

4.3. Presence of Accessory Mental Foramen:

In our study accessory mental foramen on the right side are present in 9 mandibles (6%), and on the left side 2 (1.33%) mandibles. The incidence of accessory mental foramen has been investigated in several studies, with findings demonstrating both alignment and variability when compared to the present study. Alma Soljevica et al. (2006) reported an accessory mental foramen incidence of 2.7%, which is lower than the prevalence observed in the present study. Similarly, K Udhaya et al. (2013)[13], Ronak Zarei et al[17]. (2014), Vimala et al. (2014)[18], Muna Kadel et al.[16] (2016), Kasat et al.[15] (2016), and Raja Sekhar Katikireddi et al. (2016)[19] reported lower accessory mental foramen incidences, ranging from 1.5% to 3.33% across right and left sides. Specifically, Udhaya et al.[13] (2013) found incidences of 2.22% on the right side and 3.33% on the left side, Zarei et al. (2014)[17] reported 2% on the right side and 3% on the left side, Vimala et al.[18] (2014) observed 2.6% bilaterally, Kadel et al.[16] (2016) noted 3% bilaterally, Kasat et al.[15] (2016) reported 1.5% on the right side, and Katikireddi et al.[19] (2016) found 2% on the right side. These consistently lower incidences compared to the present study may reflect variations in sample populations, imaging techniques, or diagnostic criteria used to identify accessory mental foramen.

In contrast, Devi K Sankar et al.[10] (2011) reported a higher accessory mental foramen incidence of 8.9%, surpassing the findings of the present study. This discrepancy may be attributed to differences in methodological approaches, such as the use of advanced imaging modalities or broader inclusion criteria for identifying accessory mental foramen, which could increase detection rates. Studies by Rajani Singh et al. (2010)[20], Virendra Budhiraja et al. (2012)[7], and Ritika Patel et al.[21] (2015) reported accessory mental foramen incidences that align closely with the present study. Singh et al. (2010) found incidences of 5% on the right side and 8% on the left side, Budhiraja et al.[7] (2012) reported 6.6% bilaterally, and Patel et al.[21] (2015) observed 6.45% on the right side and 1.61% on the left side. The congruence of these findings with the present study suggests a degree of consistency in accessory mental foramen prevalence within certain populations or methodological frameworks. Significance of accessory mental foramen: The presence of nerve fibers in accessory foramina may be significant in the effectiveness of local anesthesia following a routine inferior alveolar nerve block. Blood vessels present in accessory foramina can be a cause of intraosseous hemorrhages during implant procedures.

B. **Metric Parameters:**

4.4. Size of Mental Foramen:

In present study horizontal diameter of mental foramen on the right side ranges from1.03-5.12 mm and on the left side 1.01- 4.24 mm and the vertical diameter of mental foramen on the right side ranges 1.05- 4.67 mm and on the left side 1.01- 4.13 mm. The mean, standard deviation, median and p-value are presented in table numbers 4 & 5. Alma Voljevica et al.[6] (2006) reported mean vertical diameters of 1.71 mm on the right side and 1.69 mm on the left side and mean horizontal diameters of 2.56 mm on the right side and 2.41 mm on the left side. These values align closely with the present study, suggesting consistency in the morphometric profile of the mental foramen across comparable populations or methodologies. Similarly, Deepa Rani Agrwal et al.[22] (2011) found mean horizontal diameters of 3.33 mm on the right side and 3.25 mm on the left side and mean vertical diameters of 2.15 mm on the right side and 2.13 mm on the left side, which also corroborate the present study's findings. The agreement between these studies and the current results may reflect standardized measurement techniques or similar population characteristics.

In contrast, Virendra Budhiraja et al.[7] (2012) reported higher mean values, with vertical diameters of 2.61 ± 0.17 mm on the right side and 2.53 ± 0.14 mm on the left side, and horizontal diameters of 5.19 ± 0.24 mm on the right side and 5.12 ± 0.28 mm on the left side. These values exceed those observed in the present study, potentially due to differences in imaging modalities, such as the use of cone-beam computed tomography versus conventional radiography, which may offer greater precision in detecting foramen boundaries. Similarly, Hussain Sahib Shaik et al. (2012) reported mean horizontal and vertical diameters of 3.91 mm and 2.50 mm, respectively, with

standard deviations of 0.82 mm and 0.61 mm, which are higher than the present study's findings but show comparable variability, as indicated by the standard deviations aligning with the current results. K Udhaya et al. (2013) reported mean vertical and horizontal diameters on the left side of 2.52 ± 0.87 mm and 2.95 ± 0.68 mm, respectively, and on the right side of 2.86 ± 0.83 mm and 2.28 ± 0.71 mm, respectively. Priya P Roy et al. (2014) found the mean horizontal diameters of 3.01 mm on the right side and 3.22 mm on the left side and mean vertical diameters of 2.24 mm on the right side and 2.11 mm on the left side, with the vertical diameter findings aligned with the present study, while the horizontal diameters are slightly higher. Rahul Rai et al. (2014) reported mean horizontal diameters of 2.63 ± 0.85 mm on the right side and 2.61 ± 0.85 mm on the left side, and vertical diameters of 2.33 ± 0.64 mm on the right side and 2.29 ± 0.60 mm on the left side, which closely match the present study, further supporting consistency in certain morphometric parameters across studies.

4.5. Location of mental foramen in relation to mandibular parameters.

The Location of the mental foramen position relative to mandibular parameters, including the symphysis menti, posterior border of the mandible, alveolar margin, and base of the mandible.

a. Distance from Symphysis Menti to Anterior Margin of Mental Foramen:

Several studies align closely with the present study's findings on the mean distance from the symphysis menti to the anterior margin of the mental foramen. Alma Voljevica et al.[6] (2006), Deepa Rani Agarwal et al.[22] (2011), Rakhi Rastogi et al.[11] (2012), Hussain Sahib Shaik et al.[24] (2012), K Udhaya et al.[13] (2013), Ritika Patel et al.[21] (2015), Rakesh Kumar Shukla et al. [8](2015), Muna Kadel et al.[16] (2016), and Samreen Siraj Bala et al. (2017)[23] reported mean distances ranging from [24].19–26.71 mm, closely matching the present study. Conversely, Singh et al.[20] (2010) and Devi K Sankar et al. (2011)[10] reported higher values (29.3–30.6 mm and 27.2–27.7 mm, respectively), while Ajay Parmar et al.[12] (2013) and Rahul Rai[14] et al. (2014) reported lower values (22.23–23.30 mm). These differences may reflect variations in population demographics or measurement techniques, such as radiographic modalities.

b. Distance from Posterior Margin of Mental Foramen to Posterior Border of Mandible:

The present study found mean distances of 62.54 mm on the right side and 63.06 mm on the left side, with no statistically significant difference (p=0.213). Studies by Rakhi Rastogi et al. (2012), Ajay Parmar et al. (2013), K Udhaya et al.[13] (2013), Rahul Rai et al. (2014), Ritika Patel[11] et al. (2014), Rakesh Kumar Shukla et al.[8] (2015), and Muna Kadel et al.[16] (2016) reported mean distances (61.3–66.68 mm) that align with the present study. In contrast, Alma Voljevica et al.[6] (2006) reported lower values (58.68–59.34 mm), and Devi K Sankar et al.[10](2011) reported higher values (70.7 mm bilaterally). Standard deviations in most studies (4.06–5.38 mm) are comparable to the present study's (5.23–6.18 mm), suggesting similar measurement variability.

c. Distance from Alveolar Margin to Upper Margin of Mental Foramen:

The present study observed mean distances of 10.81 mm on the right side and 10.74 mm on the left side, with no significant difference (p=0.4073). Rakhi Rastogi et al.[11] (2012), Ajay Parmar et al.[12] (2013), Rahul Rai et al.[14] (2014), Rakesh Kumar Shukla et al.[8] (2015), and Samreen Siraj Bala et al.[23] (2017) reported similar values (10.26–11.48 mm), while Oliveira Junior et al.[9] (2009), Hussain Saheb Shaik et al.[24] (2012), and K Udhaya et al.[13] (2013) reported slightly higher values (12.02–13.84 mm). Devi K Sankar et al.[10] (2011) and Alma Voljevica et al.[6] (2006) reported notably higher values (13.7–16.4 mm), possibly due to differences in imaging resolution or anatomical variations.

d. Distance from Lower Margin of Mental Foramen to Base of Mandible:

The present study found mean distances of 12.48 mm on the right side and 12.50 mm on the left side, with no significant difference (p=0.4515). Alma Voljevica et al. [6](2006), Oliveira Junior et al.[9] (2009), Deepa Rani Agarwal et al. [22](2011), Hussain Saheb Shaik et al.[24] (2012), K Udhaya et al. [13](2013), Rahul Rai et al.[14] (2014), Ritika Patel et al.[21] (2015), Rakesh Kumar Shukla et al.[8] (2015), Muna Kadel et al.[16] (2016), and Samreen Siraj Bala et al.[23] (2017) reported values (10.69–13.38 mm) that closely align with the present study. Devi K Sankar et al.[10] (2011) and Rakhi Rastogi et al. [11](2012) reported higher values (14.3–16.5 mm), while Ajay Parmar et al. [12](2013) reported lower values (10.7 mm bilaterally). Standard deviations (1.23–4.83 mm) are generally consistent with the present study's (1.91–1.91 mm).

CONCLUSION

Our study reinforces the critical importance of a thorough understanding of the mental foramen's anatomy, including its location and the potential presence of accessory mental foramina. This knowledge is indispensable for clinicians performing anesthetic blocks in the lower anterior teeth region and for surgeons aiming to preserve the integrity of the mental nerve during surgical interventions. Accurate localization of the mental foramen is essential to prevent nerve damage, which can lead to temporary or permanent sensory alterations, including thermal and tactile changes. The high prevalence of oval-shaped mental foramina observed in our study suggests that this is a common anatomical feature in south-indian population. This information is valuable for surgeons as they plan their approaches to the mental region of the mandible. The presence of accessory mental foramina, although less frequent, is also a significant consideration, as these structures can potentially house additional nerve branches that may be at risk during surgical procedures. We recommend that future studies expand on this research by including larger sample sizes and investigating the influence of age, sex, and ethnicity on the mental foramen's morphometry.

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

INCIDENCE OF ACCESSORY MENTAL FORAMEN AND ITS CLINICAL SIGNIFICANCE – A REVIEW

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ABSTRACT

The accessory mental foramen (AMF) is a type of mandibular variation which transmits extra neurovascular branches, a fact with great importance in dental and surgical procedures. Its range and features do vary between populations, which is why broad based knowledge is required to which out to avoid issues like nerve injury or anesthesia failure. We did this review to look at the anatomical features, that is size and location of AMFs, their global prevalence, and what clinical use they have, and how they play a role in dental practice and forensics.

A systematic literature search was conducted using PubMed, Scopus, and Web of Science databases for studies published in the past five years. Keywords included: Accessory mental foramen (AMF), Bucal foramen, Mental foramen, Mandible, Dental surgery. The review was conducted in accordance with the PRISMA guidelines.

We did a thorough search of the literature looking at AMF prevalence, morphology and clinical implications across many populations. We looked at studies done on dry mandibles, radiographic reports and at what Cone-Beam Computerized Tomography (CBCT) shows. Also, we put together key studies which we looked at to present prevalence rates, types of variation in anatomy and diagnostic methods. AMF's range from 1% to 10% worldwide, we see higher rates in South Asian groups at 6 to 8.9% as compared to Iranian at 2 to 3% or Bosnian at 2.7%. AMFs are small, oval and are found near the first molar or second premolar. CBCT does a better job in detecting these as compared to panoramic x rays, also morphological studies give very precise measurements although may not see the smaller AMFs. The presence of AMFs reports anesthetization failure, nerve injury or bleed out during dental procedures and play a role in forensics and anthropology. AMFs are important anatomical variants that require attention in dental and surgical planning. CBCT is the best for accurate detection. By being aware of the AMF's prevalence and what they look like we see better results in care and less complications. Future research should standardize diagnostic criteria and explore demographic influences to improve clinical and forensic applications.

Keywords**:** Accessory mental foramen (AMF), Bucal foramen, Mental foramen, Mandible, Dental surgery

INTRODUCTION

1.1. Mental foramen and anatomical importance: The mental foramen, an important anatomical structure present on the anterior surface of the mandible, is the main pathway for the mental nerve and vessels, which furnish the innervation to the lower lip, labial mucosa, and chin skin [1]. Differences in the mental foramen's position, size, and shape have been widely described in various populations, underlining the necessity of a thorough knowledge of its anatomical features regarding surgical and diagnostic interventions [2]. Aside from the usual single mental foramen, accessory mental foramina can occur, which are additional openings that carry minor nerve branches and vessels [3].

1.2. Accessory mental foramen: although less common than its major counterpart, takes on significant

clinical importance owing to the ability of such structures to affect the success and safety of many dental and surgical procedures [3]. The prevalence of accessory mental foramina shows significant variation among different populations, highlighting the role of genetic andenvironmental determinants in their occurrence [2]. Its presence can result in neurosensory complications during operations, open reduction of mandibular fractures, or implant operations [4].

The precise identification and characterization of accessory mental foramina are important for avoiding iatrogenic damage to the mental nerve and related neurovascular structures and ensuring reduced postoperative morbidity and improved patient outcome [5]. This review is intended to present a broad overview of the AMF, including its anatomical features, prevalence, clinical relevance, and diagnostic options. Through review of available literature and emphasizing crucial considerations for clinical practice, this review hopes to raise dental professionals' awareness about the significance of identification and management of accessory mental foramina in respect of different clinical situations [3]. This article emphasizes the anatomical variations of the AMF, its clinical relevance in dental and surgical treatment, and proper imaging methods for its detection.

2. Anatomy of the Accessory Mental Foramen

2.1. Accessory Mental Foramen: It is described as an extra opening which occurs close to the main mental foramen, usually smaller in diameter, which carries accessory branches of the mental nerve and blood vessels.

The AMF is characterized as a foramen of an accessory bony canal with its origin in the mandibular canal [5]. Identification of an AMF is important during surgical planning to prevent nerve damage and facilitate effective anesthesia [3]. The dimensions, shape, and correct location of the mental foramen are variable [5]. The distance of the AMF from the mental foramen is between 0.67 and 6.3 mm, and the transverse diameter is between 0.81 and 3.9 mm [5].

2.2.Prevalence and anatomical variations: Prevalence of accessory mental foramina as reported in the literature varies greatly among studies and populations from 1% to 10% [5]. The reasons may lie in variations in sample size, study design, and population groups considered.

It is important to observe that genetic, environmental, and ethnic factors may be held accountable for the variation in location and frequency of the mental foramen and the accessory mental foramina in various populations. Accessory mental foramina can either be unilateral or bilateral and may be placed anterior, posterior, or below the main mental foramen. The size of accessory mental foramina is usually smaller than that of the primary mental foramen, measuring between less than 1 mm to a few millimeters. The location of the mental foramen in relation to the teeth is variable, with the most frequent location in line with the second premolar [6]. It can also be located between the first and second premolars, or even distal to the second premolar [7,8].

2.3. Neurovascular Supply:

Accessory mental foramina convey smaller branches of the mental nerve and vessels, which supply sensory innervation to the lower lip and labial mucosa [3].

These accessory nerve branches could overlap in their distribution with the main mental nerve, and hence there could be differences in sensory perception and efficacy of anesthesia.

The mental nerve arises from the inferior alveolar nerve, which is a branch of the mandibular nerve (V3), and leaves the mandible by passing through the mental foramen. The mental nerve provides sensory innervation to the lower lip, chin, and labial gingiva. The mental nerve also branches out further into finer nerves that pass out through the AMF. Accessory mental foramina can create a more variable and complex sensory innervation pattern in the anterior mandible.

3. Prevalence and Distribution

3.1. Global Prevalence: Reported prevalence of accessory mental foramina in various populations (e.g., South Indian, Mongoloid, Caucasian) varies between 1% and 10%. These differences may be due to variations in study designs, sample sizes, imaging modalities, and population genetics. Prevalence of accessory mental foramina also differs between various ethnic and geographical populations. Genetic ancestry, environmental factors, and diet may be among the contributing factors for these population-based variations. For example, research has proven that the prevalence of accessory mental foramina is increased in some populations, including African Americans and Native Americans, relative to Caucasians [9]. In particular, the frequency among French populations is approximately 2.6%, 1.4% among American Whites, 5.7% among American Blacks, 3.3% among Greeks, 1.5% among Russians, 3.0% among Hungarians, 9.7% among Melanesians, and 3.6% among Egyptians [10].

3.2. Age and Gender Factors: Limited evidence indicates that the frequency of accessory mental foramina is different with respect to age and sex. More research is required to determine the correlation between these demographics and the frequency of accessory mental foramina. The most frequent location of the mental foramen was observed to be along the long axis of the second premolar followed by between the second premolar and first molar [11].

Among Caucasians, the mental foramen is in front of the second premolar [2]. Among Mongoloids, the mental foramen is more anterior than in Caucasians [2]. There are gender variations in mental foramen location as well, with some of the studies reporting more anterior location in males as opposed to females. Based on very few studies with large samples, roughly one out of fifteen people possess AMF [12]. Moreover, males are slightly more likely to have AMF than females[12]. On the other hand, according to other research, there is no significant gender variation [12].

3.3. Bilateral and Unilateral Occurrence: Frequency of accessory mental foramina on right vs. left sides It has been observed that accessory mental foramina can occur unilaterally (on one side of the mandible) or bilaterally (on both sides). Most cases are unilateral, with bilateral cases being relatively rare. Understanding the frequency of unilateral versus bilateral occurrence is essential for surgical planning and diagnostic accuracy. Bilateral occurrence of AMF was noted in a single case [9]. A few studies did not reveal the presence of bilateral AMF [9]. Accessory mental foramina can be symmetrical or asymmetrical as regards size, shape, and location. Asymmetry can be more common because of developmental differences or environmental factors.

3.4. Demographic Factors: Impact of age, sex, and ethnicity on prevalence demographics like age, sex, and ethnicity can impact the prevalence and nature of accessory mental foramina. Genetic predisposition, environmental influence, and lifestyle behaviors might be responsible for differences in prevalence and form among populations. The most common location of the foramen, using the teeth as a landmark, was between the premolars in both males and females [13]. The shape of the foramen was oval with a slightly greater mean diameter in males than females. Accessory mental foramina are anomalies, and their occurrence has been established by cadaveric dissections, radiographs, and cone-beam computed tomography [9]. The incidence of AMF also differs significantly based on the population being examined. For instance, Balcioglu's 2011 research presents a 30-week-old fetus with MFA on the right side. Its incidence among the population differs based on ethnic and gender differences, ranging from 1% among the Russian people to 10% among the Arabic population [14]. AMFs are often unilateral, and they may also infrequently occur bilaterally [4,15].

4. Detection Methods

4.1. Morphological Examinations: Direct dry mandible measurement with Vernier calipers is a conventional approach to examining the morphology of accessory mental foramina. Direct measurement yields precise data on foramina size, shape, and position. Radiographic imaging such as panoramic radiographs and cone-beam computed tomography is crucial to use when detecting and describing accessory mental foramina in clinical practice [1]. Radiography enables visualization of foramina and their proximity to surrounding anatomical structures without the need for invasion. Traditional radiography provides minimal visualization of accessory mental foramina because of anatomical overlapping structures and low image resolution. Cone-beam computed tomography has higher accuracy and reliability in the detection of accessory mental foramina than traditional methods of imaging [1].

4.2. Cone Beam Computed Tomography: CBCT allows for detailed 3D visualization for the localization of accessory mental foramina. CBCT imaging enables accurate three-dimensional localization of accessory mental foramina, which helps in surgical planning and prevents neurovascular damage [16]. CBCT offers highly accurate images with little distortion, which helps in proper evaluation of the size, shape, and position of the foramina. The ability of CBCT to perform multiplanar reconstruction is vital, providing extensive visualization of these foramina in axial, coronal, and sagittal planes, critical to enhancing diagnostic accuracy and proper surgical preparation.

4.3. Identification Challenges: Clinical palpation and visualization are of limited use in identifying accessory mental foramina as they are small and can be variable in position. Accessory mental foramina may be hard to visualize clinically or to palpate on a routine dental examination, resulting in missed diagnoses. Misinterpretation of radiographs and anatomical variation may be issues in the precise identification of accessory mental foramina. Identification of accessory mental foramina from other anatomical structures or radiographic artifacts would require meticulous assessment and clinical correlation. Imaging modalities are invaluable in identifying these critical structures precisely[17]. The combination of panoramic radiography with higher-end imaging modalities like CBCT augments the accuracy in detecting the mental foramen location, setting the platform for automated segmentation procedures that enhance diagnostic ability [18].

5. Clinical Implications

5.1. Dental Anesthesia: Effect on inferior alveolar nerve block efficiency: Accessory mental foramina can affect the efficiency of inferior alveolar nerve blocks, causing incomplete anesthesia and failure of the procedure. Information regarding the exact location and anatomy of accessory mental foramina is vital to attain efficient nerve blockade in the region. Anesthesia failure in surgical procedures can occur because of a lack of knowledge about AMF [19]. The position of the mental foramen is a significant landmark in obtaining effective anesthesia [20]. Whereas if an AMF lies even more distally from the central

foramen, either anteriorly or posteriorly, an independent injection may be required to provide sufficient anesthesia [1].

5.2. Surgical Considerations: Surgical intervention of the mental area of the mandible, including implant placement or periapical surgery, risks intraosseous hemorrhage and nerve damage owing to the existence of accessory mental foramina[1]. Special preoperative planning through radiographic imaging is critical to prevent injury to the accessory mental nerve during surgery. Mental foramen positional variations may result in nerve injury during surgery, which would manifest as sensory alteration and neuropathic pain. Preoperative radiographic evaluation is significant in determining the
relationship between the tooth and inferior alveolar nerve [21]. Familiarity with the arterial supply and navigated implant placement of the mandibular symphysis can prevent these life-threatening emergencies [22]. Careful evaluation with CBCT is important for the prevention of possible nerve injury and complications [23]. Identification of variations in anatomy such as AMF is important for the prevention of neurovascular complications from dental procedures, including implantation surgery and nerve block. The accessory mental foramina seen approximately 3 to 5 percent and an average diameter of 0.3 mm [24]. Anatomical variation, such as AMF, seen in CBCT has a great impact in surgical planning [5]. CBCT images help determine a safe zone for bone harvesting and implantation by assessing the traits of intra bony nerve canals [25].

5.3. Forensic and Anthropological Applications:

Accessory mental foramina have racial differences regarding incidence and morphology, hence useful in racial determination and forensic investigation. Accessory mental foramina may be used as supplementary anatomical landmarks in forensic and anthropological investigations of skeletal remains. Changes in foraminal size and position can shed light on population affinities and evolutionary ties [5].

Research on accessory mental foramina helps us understand the diversity of the human skeleton and its implications in different domains.

6. Comparative Analysis Between Studies of Accessory Mental Foramen:

A systematic literature search was conducted using PubMed, Scopus, and Web of Science databases for studies published in the past five years. Keywords included: Accessory mental foramen (AMF), Bucal foramen, Mental foramen, Mandible, Dental surgery. The review was conducted in accordance with the PRISMA guidelines (figure 1).

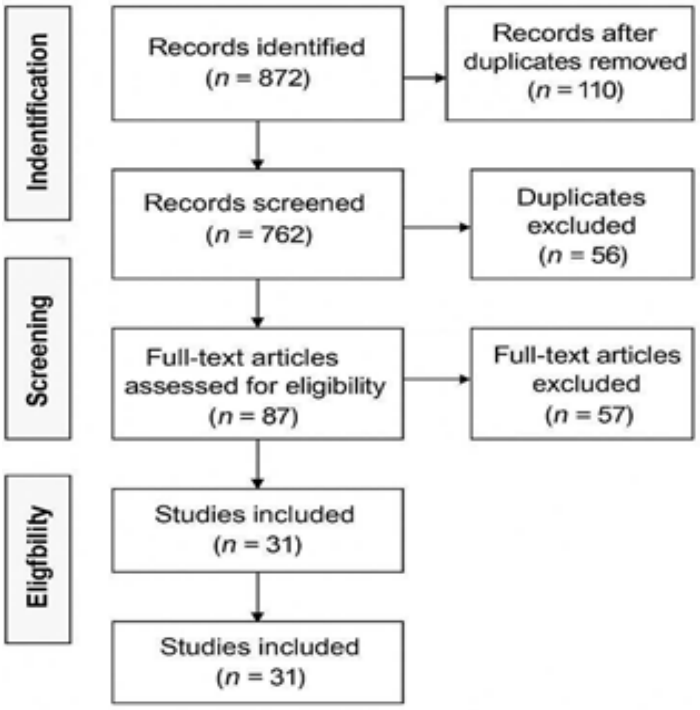


Figure 1. Bending, water sorption, and staining of custom-block, PM MA-disk and conventional PMMA [11].

The AMF is a clinically important mandible anatomical variation, possibly containing extra neurovascular structures that affect dental treatments. Several studies have investigated its prevalence and morphology across diverse populations. Voljevica et al. (2006) analyzed dry human mandibles in a Bosnian population, reporting an AMF prevalence of 2.7% bilaterally, with AMFs typically smaller and oval-shaped, located inferior or posterior to the primary mental foramen [26]. Sankar et al. (2011) studied 150 dry mandibles from South Andhra, India, and observed a greater AMF prevalence of 8.9%, frequently located close to the first molar, indicating regional differences [27]. Singh et al. (2010) described AMF prevalences of 5% on the right side and 8% on the left side in an Indian sample with variation in size and distance from the primary mental foramen [28]. Budhiraja et al. (2012) recorded 6.6% bilateral AMF frequency in mandibles of North Indians, with AMFs defined as minute, ovular apertures [29]. Patel et al. (2015) reported AMF frequencies of 6.45% on the right and 1.61% on the left side among a Gujarati population, noting asymmetry [30]. Udhaya et al. (2013) reported prevalences of 2.22% on the right side and 3.33% on the left side in Tamil Nadu, with the AMFs being mostly below the second premolar or first molar [31]. Zarei et al. (2014) found incidences of 2% on the right side and 3% on the left side in an Iranian population based on imaging methods [32]. Vimala et al. (2014) observed a 2.6% prevalence of bilateral AMF in North India [33], whereas Kadel et al. (2016) reported a 3% bilateral incidence among a Nepalese population [34]. Kasat et al. (2016) reported an incidence of 1.5% on the right side for AMF in an Indian population [35], and Katikireddi et al. (2016) observed a 2% incidence on the right side in South India [36].

Table 1. The prevalence and morphological findings of AMFs from these studies

Study	Population	Sample Size	AMF Prevalence on the right side	AMF Prevalence on the left side	Morphology and Location
Voljevica et al. (2006) [26]	Bosnian	Not specified	2.7% (bilateral)	2.7% (bilateral)	Small, oval, inferior/posterior to primary mental foramen
Sankar et al. (2011) [27]	South Andhra, India	150	8.9% (bilateral)	8.9% (bilateral)	Oval, near first molar
Singh et al. (2010) [28]	Indian	50	5%	8%	Variable size, near primary mental foramen
Budhiraja et al. (2012) [29]	North Indian	150	6.6% (bilateral)	6.6% (bilateral)	Small, oval openings
Patel et al. (2015) [30]	Gujarat, India	Not specified	6.45%	1.61%	Oval, asymmetric distribution
Udhaya et al. (2013) [31]	Tamil Nadu, India	Not specified	2.22%	3.33%	Oval, below second premolar or first molar
Zarei et al. (2014) [32]	Iranian	Not specified	2%	3%	Oval, near primary mental foramen
Vimala et al. (2014) [33]	North Indian	Not specified	2.6% (bilateral)	2.6% (bilateral)	Small, oval openings
Kadel et al. (2016) [34]	Nepalese	Not specified	3% (bilateral)	3% (bilateral)	Oval, near first molar
Kasat et al. (2016) [35]	Indian	Not specified	1.5%	Not reported	Small, oval, right sided
Katikireddi et al. (2016) [36]	South Indian	Not specified	2%	Not reported	Oval, near first molar

6.1. Methodological Variations: Study Design Variations

Prevalence and morphology of AMFs reported vary widely because of the differences in study design, such as sample size, method of detection, and study population. Sample sizes differed from 50 mandibles in Singh et al. (2010) to 150 in Sankar et al. (2011), Budhiraja et al. (2012), and the South Indian study [27–29]. Larger samples improve statistical validity but can be population-specific biases. Most of the studies employed dry mandible analysis using Vernier calipers for direct measurement (for example, Voljevica et al., 2006; Udhaya et al., 2013; South Indian study) [26,31], providing accuracy but possibly overlooking smaller AMFs because of dependence on naked-eye observation. Conversely, imaging studies, including Zarei et al. (2014), utilized cone-beam computed tomography (CBCT) or panoramic radiographs, which enhance the detection of tiny foramina but add variability because of variability in imaging resolution or slice thickness [32]. For instance, CBCT's greater sensitivity is likely responsible for higher AMF detection rates in some papers over dry mandible analysis. Population groups also varied, with studies examining ethnic groups (e.g., South Indian, Bosnian, Iranian, Nepalese), and possibly indicating genetic or craniofacial determination of AMF prevalence[26,27,31–34.] Diagnostic criteria for AMFs also varied; some needed evidence of a neurovascular bundle (e.g., Budhiraja et al., 2012) [29], whereas others identified AMFs by the presence of foramina only (e.g., Kadel et al., 2016) [34]. Such sample size, detection technique, and diagnostic criteria differences make direct comparisons problematic and suggest the necessity of standardized methods in AMF studies.

6.2. Consistency and Variability:

Despite methodological differences, there are a few consistent trends for AMF studies. The prevalence of AMF is consistently lower than that of the main mental foramen, between 1.5% and 8.9% [26]. South Indian cohorts among South Asian populations have been reported to have higher incidences of AMFs (6–8.9%) [27,31] than Nepalese (3%) [34] or Iranian (2–3%) populations [32], indicating possibly regional or genetic factors. Morphologically, the AMFs are invariably smaller and oval, found usually inferior or posterior to the main mental foramen, commonly adjacent to the first molar or second premolar [27–30]. Asymmetry is the common finding, and in studies such as Patel et al. (2015) (6.45% right vs. 1.61% left), Singh et al. (2010) (5% right vs. 8% left), and the South Indian study (6% right vs. 1.33% left), side-specific differences were reported [3,5,12], but the developmental or biomechanical reasons are unknown.

Prevalence and site discrepancies are significant.

Sankar et al (2011) also reported the maximum AMF prevalence (8.9%) [27] well above Voljevica et al (2006) (2.7%) [26] and Kasat et al (2016) (1.5% right) [35]. These differences could be due to methodological variations, like analysis of mandible drying vs imaging, or anatomical characteristics specific to populations. South Indian populations, for example, could have greater AMF prevalence because of peculiar mandibular morphology [27]. Variability in location is also seen, whereas most of the studies place AMFs adjacent to the first molar or second premolar, Udhaya et al. (2013) observed variations with regards to distance from the primary mental foramen, perhaps due to variable measurement methods or anatomical definitions [31]. The clinical relevance of AMFs is repeatedly highlighted, with research indicating their effects on the efficacy of anesthesia (owing to extra nerve branches) and intraoperative hemorrhage risks during the implantation procedure [27,29].

Summary of major studies on AMF prevalence and morphology Prevalence rates of accessory mental foramina have been reported to vary from 1% to 10% of the population [37]. Differences in sample populations, imaging modalities, and diagnostic criteria are likely causes of such variations. The prevalence of double mental foramina was reported to be 4.2% by a CBCT scan study. Varying prevalence rates of AMF occur among different populations. Variables that influence these include genetics, ethnicity, and environmental factors.

7. Impact on Clinical Practice: Understanding the prevalence and nature of accessory mental foramina is crucial for evidence-based clinical decision-making. Clinicians need to consider the presence of accessory mental foramina when they plan surgical or anesthetic interventions in the mental area. This puts the imaging modalities in dental and surgical interventions into perspective. The existence of AMF can influence the success of local anesthesia, making the bleeding risk greater during surgical interventions [2]. Accessory mental foramina cannot be detected on radiographs and usually go undetected on clinical examination. Our research supports the value of knowledge about the anatomy of the mental foramen, including the possibility of having accessory mental foramina [2]. Accessory mental foramina are anatomical variations detectable by cone-beam computed tomography [37]. Knowledge about accessory mental foramina can assist dental clinicians in enhancing the outcome of treatments and minimizing complications during dental procedures. The size and number of accessory mental foramina differ from person to person [12]. Accessory mental foramina may be identified through cone-beam computed tomography to diagnose radiomorphometric and epidemiological parameters.

8. CONCLUSION

The accessory mental foramen is a widespread anatomical variation of the mandible that has an impact rate of 1% to 10% among various populations based on genetic, ethnic, and environmental conditions. This systematic review underscores the vital clinical significance of AMFs in dental and surgical interventions, especially in procuring proper anesthesia and avoiding neurovascular complications during such interventions as implant placement and mandibular surgery. The heterogeneity of AMF prevalence, morphology, and location, usually smaller, more oval, and located closer to the first molar or second premolar, emphasizes the need for accurate preoperative imaging, with CBCT becoming the gold standard due to its superior high-resolution, three-dimensional visualization. Despite methodological variations between studies, e.g., sample size, detection methods, and diagnostic criteria, recurrent patterns validate the clinical significance of AMFs and the necessity for uniform research protocols to maximize comparability. Identification of AMFs is crucial for dental professionals to enhance treatment results, reduce iatrogenic trauma, and increase patient safety. In addition, AMFs have the potential for applications in forensic and anthropological analyses, providing insights into population diversity and the identification of the skeleton. Future studies must address clarifying the demographic factors, including gender and age, as well as creating automated detection devices that can incorporate AMF identification into clinical practice easily, eventually promoting evidence-based dentistry.

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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

AI-DRIVEN VIRTUAL ASSISTANTS IN DENTISTRY:ENHANCING PATIENT CARE WORKFLOW EFFICIENCY

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ABSTRACT

Background: Dental practice management demonstrates positive potential through AI-powered virtual assistants since these systems perform administrative tasks while enhancing patient consultations. Evidence about their effect in actual dental clinics is still insufficient to measure.

Objective: This observational prospective research measured the outcomes of AI-based virtual assistants on dental care processes and practice workflow in dental offices.

Methods: The studybase included 168 dental practices which fulfilled the requirements regarding their use and stability of AI-based virtual assistants. Data collection took place at baseline assessment during Months 0-3 and after three months during Months 4-6 followed by six months in Months 7-9. The quantitative assessment involved patient satisfaction ratings as well as treatment success rates and appointment scheduling speeds and time spent handling administrative duties and the entire workflow efficiency. The study obtained qualitative data by conducting interviews which underwent thematic analysis.

Results: The patient satisfaction scores together with treatment success rates and appointment scheduling efficiency and administrative task completion time and overall workflow efficiency improved significantly at both three-month and six-month follow-ups ($p < 0.05$). The analysis used regression to show how patients reacted to using AI virtual assistants but it took into consideration resource duration and practice size and location and starting patient satisfaction scores (adjusted R-squared: 0.573).

Conclusion:AI-powered virtual assistants enhance both dental patient care procedures and clinical workflow operation in dental offices according to this study. Dental practices can optimize their practical operations while improving patient satisfaction to determine the proper integration techniques for dental technology in future developments.

Keywords: Artificial intelligence, virtual assistants, dentistry, patient care, workflow efficiency, observational study.

INTRODUCTION

Recent years have brought artificial intelligence (AI) technologies into different industries that revolutionized workflow operations and boosted efficiency while changing how services are delivered [1]. Medical institutions use AI-controlled virtual assistants that drive interest through their demonstrated ability to enhance clinical results while improving treatment procedures[2]. Medical practices in dentistry benefit from artificial intelligence virtual assistants to improve administrative operations and communication methods while enhancing clinic performance according to studies in the field [3].

The artificial intelligence-based virtual assistant platform includes multiple digital instruments which automate routine processes and help users make decisions and deliver natural language processing while using machine learning algorithms [4]. Virtual assistants in dental practice serve multiple purposes such as scheduling appointments, delivering patient communications and sending treatment plan reminders with administrative support capabilities [5]. The integration of AI technologies within virtual assistants allows dental practices to gain operational efficiency and ease administrative work and create better patient relationships [6,7].

Research-based evidence on the effects of AI virtual assistants on dental workflows and clinical service delivery continues to be scarce among dentistry practitioners. Real-world dental settings require thorough research to determine how well AI-driven virtual assistants function and their usability factors along with their application effects.

The study looked to fill this research gap through observations of AI virtual assistant impacts on patient care practice and management in dental workflows. The research assessed the positive aspects and barriers linked to dental practice workflows through the implementation of virtual assistants by using both quantitative and qualitative evaluation methods.

Materials and methods

Study Design and Setting

The research extended across one year to study how dental practices would be affected by AI-powered virtual assistants in terms of patient care quality together with workflow efficiency. The research data collection occurred among dental clinics that operated in urban settings and suburban areas throughout different geographic regions. The researchers employed convenience sampling to obtain willing participated practices that matched study requirements. The research method included a selection process aimed at achieving demographic and clinical environment diversity.

Sample Recruitment and Inclusion/Exclusion Criteria: Professional dental networks, conferences and online forums collectively recruited 168 dental practice sites for assessment. The research included only practices that maintained basic usage of AI-driven virtual assistants for minimum three months before the study as part of their eligibility criteria. Research institutions excluded their software when it did not contain virtual assistant technology or had insufficient usage time or refused research requirements. The study sites received reliable and consistent data because of the strict inclusion and exclusion criteria.

Baseline Assessment (Months 0–3)

Data collection through structured surveys and observational assessments and interviews took place for the first three months of the study baseline period. Causal and non-causal survey data obtained information about patient populations and scheduling procedures, care plans, office work and workflow efficiency problems. The direct assessments through observation linked with survey information revealed real-time patient journey processes as well as staff activities and technological engagement to serve as foundation for later intervention evaluations.

Intervention and Staff Training (Months 3–12):

The introduction of AI-powered virtual assistants began in participating practices after the baseline phase completed. The systems integrated the digital tools which streamlined management processes through task automation including appointments management and patient interactions among other administrative responsibilities. Staff members from dental clinics received customized training about virtual assistant functionalities. The training sessions focused on helping staff members learn to fully use technology properly in their specific workplace practices.

Data Collection Phases

Survey data collection occurred during three predetermined periods which included baseline months 0-3 as well as months 4-6 after intervention and months 7-9 six months post-intervention. Surveys together with interviews and observational assessments constituted the method for collecting data throughout all three time points. Staff and patient survey results and observations of immediate effects were recorded at three months after the implementation while six-month data evaluated both the long-term benefits of virtual assistant implementation and possible areas for improvement.

Outcome Measures

The study tracked quantitative data points that measured patient satisfaction scores together with treatment success rates and scheduling efficiency and administrative task times and overall workflow efficiency. The assessment included data points from practice performance numbers alongside feedback from patients and system-generated time data for completion timelines. The research employed qualitative assessments that monitored AI integration from staff viewpoints about advantages and drawbacks together with staff sentiments regarding system integration. Staff members participated in surveys

and interviews which allowed researchers to study technology acceptance and system usability and understand potential employment threats that AI systems could create.

Data Analysis and Ethical Considerations:

We evaluated quantitative information using IBM SPSS version 24 software which combined descriptive statistics tools with t-tests and regression models at a p value less than 0.05 for statistical significance.

The research team used thematic analysis to identify common themes present in the qualitative survey responses. The research received Institutional Review Board (IRB) approval along with participant informed consent from everyone who took part in the study. Throughout the research process the investigators maintained absolute data security measures and followed confidentiality protocols to protect ethical standards.

RESULTS

A significant enhancement in patient satisfaction emerged through time since dental practice adoption of AI-driven virtual assistants as displayed by quantitative data in Table 1.

Table 1. Comparison of Patient Satisfaction Scores Over Time

Time Point	Mean Satisfaction Score	Standard Deviation	p-value
Baseline (Months 0-3)	4.2	0.6	-
3 Months Post-Intervention (Months 4-6)	4.5	0.5	<0.001
6 Months Post-Intervention (Months 7-9)	4.7	0.4	<0.001

The patients scored higher satisfaction at follow-up than baseline since scores increased from 4.2 at baseline to 4.7 at six months post-intervention ($P < 0.001$). The data from Table 2 supports an increase in treatment success rates after the intervention which surpassed baseline numbers (92% vs. 85%, $p = 0.023$) revealing better clinical results.

Table 2. Treatment Outcomes Analysis

Outcome Measure	Baseline (%)	Post-Intervention (%)	p-value
Treatment Success	85	92	0.023
Complications	12	8	0.087

Ending Table 3 demonstrates the beneficial results of the scheduling efficiency enhancement which occurred after implementing the intervention. The waiting period underwent a 5-minute reduction ($p < 0.001$) along with a decline in appointment cancellations from 8% to 5% ($p = 0.012$) thus leading to improved schedule access with fewer canceled appointments.

Table 3.Appointment Scheduling Efficiency Analysis

Metric	Baseline (Minutes)	Post-Intervention (Minutes)	p-value
Wait Times	15	10	<0.001
Cancellations	8%	5%	0.012

Research results in Table 4 demonstrated the significant reduction of administrative time needed to finish different tasks ($p < 0.05$) for patient registration, insurance examination, billing functions and maintaining medical records.

Medical practice benefits from AI-driven virtual assistance because it brings improved administrative process management and enhanced operational efficiency.

Table 4. Administrative Task Completion Time Analysis

Task	Baseline (Minutes)	Post-Intervention (Minutes)	p-value
Patient Registration	20	15	<0.001
Insurance Verification	12	8	0.003
Billing	18	12	<0.001
Record-Keeping	25	20	0.021

Moreover, Table 5 highlights improvements in overall workflow efficiency post-intervention, with increases in patient flow, staff productivity, and resource utilization (all $p < 0.05$). Practice operations become more efficient while resource management becomes optimized after the deployment of virtual assistants.

Table 5. Overall Workflow Efficiency Analysis

Metric	Baseline (%)	Post-Intervention (%)	p-value
Patient Flow	7.5	8.8	0.009
Staff Productivity	80	85	<0.001
Resource Utilization	90	92	0.032

Table 6 shows the duration of virtual assistant utilization as an essential parameter for patient satisfaction scores with a strong positive association ($\beta = 0.345$, $p < 0.001$). This result indicates that extended system usage increases the level of patient satisfaction. The analysis established that practice size as well as initial patient satisfaction score and location were additional elements (all $p < 0.05$) that correlated with patient satisfaction alongside virtual assistant use duration ($\beta = 0.345$, $p < 0.001$).

Table 6. Regression Analysis of AI-Driven Virtual Assistant Utilization on Patient Satisfaction Scores

Predictor Variable	Beta Coefficient	Standard Error	t-value	p-value
Duration of Utilization	0.345	0.078	4.421	<0.001
Practice Size (Number of Dentists)	0.128	0.052	2.462	0.015
Urban vs. Suburban Location	-0.052	0.061	-0.852	0.396
Initial Patient Satisfaction Score	0.241	0.067	3.597	0.002

Adjusted R-squared: 0.573

The qualitative analysis in Table 7 identified four main themes that stemmed from thematic analysis of dental practices: improved communication between staff and patients, streamlined appointment scheduling, lower administrative workload and resistance to change and technical problems which presented challenges. The identified themes helped unveil significant qualitative implications of virtual assistants on dental practice operation.

Table 7. Key Themes from Qualitative Analysis

Themes	Description
Improved Communication	Enhanced patient-provider communication through virtual assistants
Streamlined Scheduling	Simplified appointment scheduling processes
Reduced Administrative Burden	Decreased workload for administrative tasks
Resistance to Change	Initial reluctance to adopt new technology
Technical Issues	Challenges related to software glitches or system compatibility

The evaluation of staff opinions in Table 8 shows helpful reactions to technical systems and recognized benefits yet staff members express concerns about job loss risks and continue seeking training opportunities. Staff concerns and professional support need direct attention because it will help dental practices receive the full benefits from AI-driven virtual assistants.

Table 8. Staff Attitudes and Experiences

Findings	Description
Satisfaction with Technology	Positive feedback on ease of use and perceived benefits
Concerns about Job Displacement	Apprehension regarding potential impact on job roles and responsibilities
Need for Ongoing Training	Recognition of the importance of continued education and support

DISCUSSION

The healthcare industry shows marked interest in using artificial intelligence to create virtual assistants for dental practice to enhance patient experiences and dental workflow operations. Research findings from this study offer critical information about how AI virtual assistants affect dental practices together with patient health results.

Patient satisfaction scores increased substantially after doctors introduced AI-driven virtual assistants into their practices according to the study findings. The satisfaction levels of patients demonstrated positive growth as their mean scores improved from 4.2 at baseline to reach 4.7 marks after six months of the intervention implementation. The virtual assistants help enhance patient satisfaction by enabling efficient appointment scheduling while facilitating quick patient relations through personalized dialogues [2,6,8].

The delivery of proactive updates and automated task handling capabilities by virtual assistants produces streamlined practice operations along with shortened waiting times so patients become more satisfied [9,10]. The use of virtual assistants creates better possibilities for patient education and engagement thus enabling more active oral health management among patients[11,12].

Virtual assistant features including appointment reminders and treatment plan explanations improve the patient-provider relationship because they enhance dental practice patient trust and confidence [3,5].

AI-based virtual assistant systems introduced to dental offices support extreme workflow performance gains across different clinical areas. After implementing the intervention workflow metrics showed important improvements regarding appointment scheduling efficiency and both administrative tasks completion time and overall productivity. Virtual assistants reduced appointment waiting times from 15 minutes at baseline to 10 minutes post-intervention because they streamlined the appointment scheduling process [6,13].

Reductions in patient registration and billing completion times happened at dental offices thus dental staff could provide more direct care to patients. Virtual assistants automate administrative work to eliminate manual mistakes while eliminating paperwork and enhancing data precision which enables the improvement of practical operational efficiency[2,14]. The focus on high-quality patient care by dental practitioners becomes possible through these neurological advancements which result in improved productivity together with enhanced patient satisfaction [3].

The deployment of AI-driven virtual assistants produces positive effects which simultaneously enhance both patient satisfaction and workflow efficiency as well as treatment outcomes in dental practice. The investigation showed treatment successes escalated to 92% from their original 85% baseline figure after intervention occurred. Two key advantages of virtual assistants support dental provider-patient communication and patient treatment compliance and proactive follow-up activities[15,16].

The role of virtual assistants lies in connecting dental providers with their patients through time-sensitive notification systems and educational content and post-treatment guidance[5,17]. Through their role of reinforcing care instructions virtual assistants help patients adhere to therapy plans which results in improved healthcare results and decreases treatment complications[18]. Virtual assistants provide various functions which include tracking patient progress and detecting early warning symptoms to organize efficient interventions that boost treatment effectiveness and patient recovery results [8].

Factors Influencing Adoption and Implementation

Multiple variables between dental practices and virtual assistant adoption exist which could impact their implementation and utilization in dentistry practice. Staff members report resistance toward changes along with technical complications and worry about job replacement as vital barriers during qualitative research[19]. Healthcare professionals show initial reluctance toward adopting new technology due to their unfamiliarity regarding its function and their belief that workflow changes will negatively impact operations and their doubts about virtual assistance system reliability.

The implementation of virtual assistants in dentistry requires active measures which include staff training programs combined with workflow integration support and solutions for ensuring worker safety against job displacement changes in roles. Staff training through continuous educational programs enables medical personnel to use virtual assistant features efficiently and ultimately enhances their utilization of AI technology for delivering dental healthcare. Dental staff along with practitioners must clearly receive information about how virtual assistants can benefit patient care while improving workflows and reducing staff workload in order to achieve their support.

Limitations and Future Directions

The study delivers essential findings about artificial intelligence virtual assistants for dental patients yet multiple constraints should be noted. The research design based on observations restricted the capability to prove cause-effect relationships between the implementation of virtual assistants and observed results. Research needs to incorporate randomized controlled trials along with longitudinal studies which combine large sample populations from different geographical areas to produce strong evidence about virtual assistant benefits within dentistry.

The examination only measured quantitative elements related to patient satisfaction surveys and treatment results without extensive analysis of qualitative aspects that affect virtual assistant deployment in dental practice. Research needs to conduct comprehensive qualitative examinations for revealing the detailed workplace attitudes of dental staff regarding virtual assistant technology adoption in their clinical work environment.

CONCLUSION

Patients together with workflow efficiency experienced significant improvement in dental practice through AI-driven virtual assistants. Thorough examination of quantitative alongside qualitative information in the study showed major enhancements affecting domains from patient satisfaction results to clinical results as well as appointment bookings and administrative performance time alongside operational workflow efficiency. The research demonstrates how AI technology will transform dental practice management to give clinicians enhanced power for delivering quality healthcare with optimized operational performance. The identification of patient satisfaction score and staff attitude predictors offers important directions for deploying strategies that yield optimal support for continuous improvement.

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ORIGINAL RESEARCH

COMPARISON OF PEDIATRIC SYRUPS ON MICROHARDNESS AND SURFACE ROUGHNESS OF DIFFERENT RESIN RESTORATIONS: AN IN-VITRO STUDY

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ABSTRACT

Objective: The study objective was to compare the alterations in microhardness and surface roughness (SR) of three distinct resin-based restorations commonly employed in pediatric dentistry under continuous exposure (at baseline, 7, 14, and 21 days) to frequently utilized pediatric syrups.

Materials and methods: In this in vitro study, three pediatric syrups used as immersion agents were amoxicillin+clavulanic acid, paracetamol, and haematinic syrup. The materials evaluated were Compomer, Microhybrid, and Nanofill composite resin restorations. The study included 180 caries-free, non-restored deciduous molar teeth without developmental anomalies that were extracted due to pre-shedding mobility. The surface microhardness and SR were measured with a Vickers microhardness durometer and a contact profilometer respectively. ANOVA was used for intra- and inter-group data comparisons at a 5% significance level using SPSS software.

Results: On day 21, the antibiotic-micro-hybrid group had the highest microhardness (52.95±0.62). The compomer-hematinic group had the lowest microhardness (13.22±0.33). The SR of the compomer-paracetamol group was the highest (1.47±0.43), while the micro-hybrid-antibiotic group had the lowest (0.83±0.07). Intragroup comparisons showed significant differences ($p<0.01$), showing a decrease in material microhardness with an increased exposure period. The compomer subgroups exposed to paracetamol and haematinic and the nanofill composite exposed to haematinic syrup exhibited a significant difference ($p<0.01$).

Conclusion: The microhardness of compomer-paracetamol, compomer-antibiotic, and nanofill-paracetamol subgroups increased over the study period. The SR of the compomer and nanofill materials treated with haematinic syrup and compomer-paracetamol subgroup increased considerably.

Keywords: Microhardness, Pediatric syrups, Resin-based restorative materials, Surface roughness, Vickers hardness number.

1.INTRODUCTION

The utilization of resin-based restorative materials (RRM) has surged in prominence owing to the rising need for minimally invasive procedures and aesthetically appealing restorations for the teeth. The long-standing efficacy of these restorations is contingent upon numerous pivotal aspects, including microleakage, water solubility,

polymerization shrinkage, cavity morphology, application method, and surface topography.[1] The desire for aesthetic appearances concerning diverse dental materials is progressively expanding in pediatric dentistry.[2] The pediatric population generally receives treatment with glass ionomer cement, compomers, and RRM, with specific recommendations based on the special needs of children.[3,4] The main goal in treating teeth afflicted by dental caries is to regain their aesthetics and functioning, ensuring smooth, plaque-resistant restoration devoid of porosity.[5] RRM exhibits a success percentage of around 90% in restoring class I and II cavities in primary dentition under local anesthesia and using a rubber dam.[6] On the contrary, compomers, despite demonstrating inferior durability to wear relative to RRM, have the benefit of prolonged fluoride delivery. Nonetheless, they are linked to certain constraints, such as diminished resistance to masticatory stresses relative to composite restorations, heightened vulnerability to moisture as opposed to conventional glass ionomer cement, and heightened surface roughness (SR).[7] Research initiatives to improve resin matrices predominantly concentrate on improving physical, mechanical, and aesthetic characteristics. Concurrently, there are endeavours to diminish SR to augment wear resistance and facilitate polishing by modifying the framework of inorganic fillers, thereby attaining an effectively smooth surface to preserve the general characteristics of restorations. A rough surface, on the other hand, may result in concerns such as plaque buildup, bacterial adhesion, discolouration, gingival disorders, and the emergence of secondary caries. The longevity of restorations is greatly influenced by microhardness, which is intimately related to the material's rigidity and durability. Restorations exhibiting lower surface hardness are vulnerable to abrasions, resulting in a diminished longevity rate.[7] In addition to meeting aesthetic standards, RRM should possess good fracture or abrasion resilience and clinical endurance. Consequently, new materials are consistently manufactured, while current materials are being improved on an ongoing basis.[8] Nonetheless, convincing evidence addressing the supremacy of any particular material for restorations employed in dentistry is lacking.[9] Consequently, clinical judgments concerning the choice of materials must consider criteria that include caries activity, the application of minimally invasive procedures, cavity form, and aesthetic considerations.[10]Pediatric liquid medications are typically provided for children for various chronic needs, including analgesics, antibiotics, antiepileptics, vitamin supplements, and antitussives. Consequently, the application of these preparations over a brief duration could potentially be regarded as having extended effects. Their sugar content may lead to cariogenic effects and also exhibit erosive potential due to heightened acidity.[11-13] Acidic formulations are regarded as essential for the distribution of medication, chemical durability, physiological adaptability, and flavor improvement.[14] However, aspects such as acidic constituents, frequency and length of use, use of medicine before bedtime, intake of food, excessive viscosity, and elements that diminish salivary flow contribute to tooth erosion.[15,16] Paediatric syrups with low pH levels have already been demonstrated to induce imperfections on enamel surfaces and dental restorations, eventually leading to diminished hardness of restorations and teeth.[7] Henceforth, the study objective was to compare the alterations in microhardness and SR of three distinct RRMs commonly employed in pediatric dentistry (compomer, microhybrid, and nanofill composite resins) under continuous exposure (baseline, 7, 14, and 21 days) to frequently utilized and pediatric syrups (antibiotics, analgesic, and hematinic syrups).

MATERIALS AND METHODS

Pediatric syrup and restorative materials

The present in vitro research utilized three distinct pediatric syrups as immersion agents: an antibiotic (amoxicillin 200 mg + clavulanic acid 28.5 mg per 5 ml), Paracetamol (250 mg/5 ml), and hematinic syrup (Ferrous ascorbate (30 mg), Zinc sulfate (8 mg), Folic acid (140 mcg) per 5 ml). The medications utilized were chosen from long-term and routinely prescribed medications that pediatricians often recommend for acute or chronic conditions. The restorative materials assessed were Compomer (Dyract XP), Microhybrid composite (Ivoclar Te-Econom plus), and Nanofill composite (Filtek Supreme XT). The characteristics of pediatric pharmaceuticals and RRMs utilized in this investigation are illustrated in Tables 1 and 2.

Sample Size and Selection of Teeth

Utilizing G*Power version 3.1 software (Heinrich Heine University, Germany) for power analysis, we established that a minimum of 180 samples was necessary, with 20 samples within each subset, to attain an error margin of 0.05, an effect size of 0.30 (derived from the pilot study), and a power of 0.80 at the 5% significance level. The investigation included 180 caries-free, non-restored deciduous molar teeth, devoid of developmental abnormalities, necessitating extraction due to pre-shedding mobility. After collecting the teeth, they were preserved in a 0.5% chloramine T solution. Before application, the teeth were cleansed with pumice slurry utilizing a polishing brush attached to a low-speed handpiece.

Table 1. Summary characteristics of the composite resin restorations evaluated

Materials and their type	Composition	Manufacturer	Filler percentage
Polyacid-modified composite resin - Compomer (Dyract XP)	UDMA, TEGDMA, TMPTMA, Carboxylic acid modified dimethacrylate, butylated hydroxyl toluene, Strontium-alumino-sodium-fluoro-phosphor-silicate glass, strontium fluoride, titanium and iron oxide	Dentsply DeTrey, GmbH, Germany	47 wt% 50 vol%
Microhybrid composite (Ivoclar Te-Econom plus)	Dimethacrylate and TEGDMA, barium glass, ytterbium trifluoride, silicon dioxide and mixed oxide	Ivoclar, Vivadent, Liechtenstein	76 wt% 60 vol%
Nanofill composite (Filtek Supreme XT)(23)	Bis-GMA, UDMA, TEGDMA, Bis-EMA, zirconia/silica nanoclusters	3M ESPE AG, Seefeld, Germany	78.5 wt% 63.3 vol%

Table 2. Pediatric syrups evaluated in the study

Pediatric syrup	Generic name
Antibiotic	Amoxicillin+clavulanic acid (200 mg/28.5 mg per 5 ml)
Analgesic	Paracetamol 250 mg/5 ml
Hematinic	Ferrous ascorbate (30 mg), Zinc sulfate (8 mg), Folic acid (140 mcg) (per 5 ml)

Preparation of Specimens

For each medication, 20 specimens (8mm x 2mm) were made using use moulds. A complete set of 180 restorative materials (n=60 for each) was made following the manufacturers' specifications. Light-polymerized was employed for 20 seconds utilizing an LED (Guilin Woodpecker, Guangxi, China) with a wavelength of 420 to 480 nm and a power level of 1400 mw/cm². Sample surfaces were secured with a Mylar strip band to ensure uniformity across each material. Each specimen was polished with the Super-Snap Rainbow Technique Kit (Shofu Inc., Japan) and the One Gloss Polishing Kit (Shofu Inc., Japan). Subsequently, samples were preserved in sterile water at 37°C for 24 hours.

Specimen immersion cycles

The samples generated in each RRM were thereafter categorized into three distinct groups (n=20) according to the pediatric medication preparations to be analyzed. After the initial microhardness and SR assessments, acrylic blocks were randomly allocated to the respective medications for immersion of the specimens. Throughout the immersion sessions, the specimens were immersed for one minute, three times daily, in 10 mL of undiluted syrup preparation. To ensure that the medication solutions were homogeneous, the solutions were mixed just before immersion in each cycle. After every immersion session, the specimens were cleaned with sterile water and kept in 10 mL of artificial saliva at ambient temperature till the subsequent immersion session, following the protocol established by McKnight-Hanes and Whitford,[17] as amended by Amaechi et al.[18] The treatment was conducted for 21 days, resulting in 63 immersion cycles. The solutions and artificial saliva were replaced every day for every specimen. The surface microhardness and SR were evaluated at 7, 14, and 21 days following continual systematic repetitions of regular immersion sessions.

Microhardness Examination

The surface microhardness measurements were obtained using a Mitutoyo HV-1000 Vickers microhardness durometer, and the results were documented as Vickers hardness number (VHN). Three notches were created at various locations on every sample, maintaining a minimum distance of one mm from the proximal notch, using a weight of 100 grams for 15 seconds. The mean of the recorded triplicate readings was denoted as VHN in Kg/mm².

Surface Roughness Examination

SR measurements were obtained using a contact profilometer (SJ-201P, Mitutoyo, Japan) and were performed with a 0.8 mm cutoff point, 0.25 mm/s speed, and 0.01 µm resolution. Before evaluation, the exterior specimen surface was dried out with tissue paper, and a surface profilometer's contact guide was placed at the center of the surface. Three distinct readings in the same direction were obtained from the central area of the topmost layer of every specimen, and the average of these readings was computed. The average of the data was regarded as the mean SR of the specimens. A calibration of the profilometer was performed before every subsequent measurement cycle.

Statistical analysis

The data evaluation was performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, USA). The analysis of variance (ANOVA) was employed for both intra-group and inter-group comparisons of the collected data at a 5% significance level.

RESULTS

This study assessed the microhardness and SR of three RRMs during prolonged exposure to three pediatric syrups. Table 3 illustrates the comparative impact of pediatric syrups (antibiotic, analgesic, and hematinic syrups) on the microhardness of the materials (Compomer, micro-hybrid, and nanofill composite resins) throughout specified intervals (0, 7, 14, and 21 days). On day 21, the micro-hybrid-antibiotic group exhibited the highest microhardness (52.95±0.62 Kg/mm²), subsequently followed by the micro-hybrid-paracetamol group (51.35±0.12 Kg/mm²). The compomer-hematinic group demonstrated the lowest microhardness (13.22±0.33 Kg/mm²) on day 21 (Table 3).

Table 3. Comparison of the influence of pediatric syrups on the microhardness (Kg/mm^2) of the evaluated materials

		Days				
Resin materials	Pediatric syrup (n=20 in each group)	Baseline	7	14	21	p-value
Compomer (n=60)	Antibiotic syrup	26.47±0.93	26.01±0.74	25.22±0.83	24.96±0.37	0.000**
	Paracetamol syrup	24.55±1.43	22.49±1.81	21.38±0.23	19.79±1.05	0.000**
	Hematinic syrup	21.47±1.71	17.73±1.08	15.42±1.72	13.22±0.33	0.000**
Micro-hybrid composite (n=60)	Antibiotic syrup	53.16±0.48	53.0±0.58	53.0±0.63	52.95±0.62	0.686
	Paracetamol syrup	51.49±0.18	51.44±0.22	51.41±0.41	51.35±0.12	0.38
	Hematinic syrup	49.07±0.17	48.27±0.27	48.0±0.20	48.12±0.32	0.000**
Nanofill composite (n=60)	Antibiotic syrup	42.73±0.11	42.7±0.02	42.66±0.31	42.64±0.12	0.373
	Paracetamol syrup	42.74±1.29	42.66±0.79	42.57±1.28	41.03±0.23	0.000**
	Hematinic syrup	39.52±1.76	38.39±0.84	38.07±1.14	37.86±0.73	0.000**
		0.000**	0.000**	0.000**	0.000**	

***Highly significant*

The compomer-paracetamol group exhibited the greatest SR (1.47±0.43 μm), whereas the micro-hybrid-antibiotic group demonstrated the lowest SR (0.83±0.07 μm), then followed by the nanofill-antibiotic group (0.9±0.57 μm) on day 21 (Table 4).

The subgroup of micro-hybrid resin subjected to antibiotics (p=0.686) and paracetamol (p=0.38), and the subgroup of nanofill resin exposed to antibiotics (p=0.373), demonstrated statistically insignificant differences, indicating no reduction in the microhardness of the restorations during the assessed time intervals. The intragroup comparisons of the remaining subgroups exposed to the assessed pediatric syrups revealed statistically significant differences (p<0.01), indicating that as the duration of exposure increases, a reduction in the microhardness of the materials was observed (Table 3).

In the comparison of the role of syrups on the SR of the materials being evaluated, the compomer subgroups exposed to paracetamol and hematinic exhibited a statistically significant difference (p<0.01); similarly, the nanofill composite exposed to hematinic syrup also displayed a significant difference in SR across the evaluated time intervals which illustrates that the prolonged exposure to these syrups markedly increased the SR of restorative materials (Table 4).

Nonetheless, statistically significant differences were observed for each time period (p < 0.05) when contrasting the intergroup impact of the pediatric syrups on the microhardness (Table 3) of the materials; on the other hand, an intergroup comparison of the SR revealed statistically significant differences only at days 14 and 28 (p < 0.05) (Table 4).

Table 4. Comparison of the influence of pediatric syrups on the SR (μm) of the evaluated materials

		Days				
Resin materials	Pediatric syrup (n=20 in each group)	Baseline	7	14	21	p-value
Compomer (n=60)	Antibiotic syrup	0.88±0.53	0.91±0.94	0.94±0.03	1.04±0.07	0.804
	Paracetamol syrup	0.97±0.47	1.11±0.17	1.39±0.3	1.47±0.43	0.000**
	Hematinic syrup	1.07±0.21	1.24±0.13	1.31±0.17	1.44±0.29	0.000**
Micro-hybrid composite (n=60)	Antibiotic syrup	0.73±0.28	0.77±0.48	0.79±0.55	0.83±0.07	0.879
	Paracetamol syrup	0.82±0.07	0.83±0.11	0.9±0.97	1.13±0.6	0.292
	Hematinic syrup	1.09±0.09	1.11±0.73	1.12±0.74	1.14±0.73	0.996
Nanofill composite (n=60)	Antibiotic syrup	0.84±0.78	0.86±0.84	0.87±0.8	0.90±0.57	0.996
	Paracetamol syrup	0.91±0.73	0.93±0.07	0.97±0.89	1.04±0.28	0.905
	Hematinic syrup	1.02±0.26	1.13±0.34	1.26±0.47	1.37±0.35	0.019*
		0.185	0.066	0.02*	0.000**	

**Highly significant; *Significant; SR – Surface roughness

DISCUSSION

The microhardness and SR of the materials under investigation were significantly affected by the interactions between the material and the solution being studied. Notably, the micro-hybrid materials demonstrated the greatest microhardness, while the compomer displayed the highest SR among the restorative materials analyzed. Several research studies have yielded conflicting results about the association between enhanced microhardness and the quantity of inorganic fillers in RRMs. Few studies have indicated a positive association between elevated microhardness measurements and greater inorganic fillers in RRMs,[19,20] whereas other studies have found no significant relationship between filler quantity and the mechanical characteristics of these composite materials.[21,22] Furthermore, Yesilyurt et al.[23] emphasized that these RRMs may demonstrate substantial variations in hardness when subjected to diverse chemical conditions. Nonetheless, the outcomes of the investigation by Karatas et al.[7] did not demonstrate any correlation between the microhardness of the various RRMs examined and the quantity of inorganic filler. Our findings were in line with the prior studies[7,24] which documented statistically significant variations in the mean microhardness of the evaluated specimens.

The assessment of SR is a crucial technique for evaluating surface modifications, especially in the field of dentistry.[25] Various tools, including electron microscopes and profilometers, have been commonly employed to evaluate SR in dentistry. Over the past few decades, atomic force microscopy has emerged as a significant instrument for such purposes, providing several benefits over other techniques, such as enhanced resolution evaluations at the nanoscale, the capability to produce three-dimensional images, and greater appropriateness for assessing restorations and dental tissue.[26] Surface microhardness is a crucial measure in assessing the properties of restoration materials. It is crucial in multiple facets of dental restorations, encompassing their resistance to masticatory forces, vulnerability to abrasion, propensity for fracture and fissure formation, maintenance of matrix integrity, and likelihood of plaque deposition.[27]

The Vickers hardness tester is a widely utilized tool in dentistry for determining surface microhardness. This assessment is supported by the short tip framework of the instrument, facilitating evaluation and enabling the examination of surface hardness in an array of materials.[28] Given these benefits, Vickers hardness tester was used in this investigation. The vulnerability of RRMs to roughness is directly associated with the resin phase. RRMs formulated using urethane dimethacrylate (UDMA) exhibit diminished surface hardness and decreased roughness relative to those formulated with bisphenol A-glycidyl methacrylate (Bis-GMA). The micro-hybrid materials displayed the lowest SR measurements owing to the disparity in the amount of filler per volume of RRM. The inclusion of pre-polymerized fillers in the micro-hybrid composite materials, together with the inclusion of fillers like glass, ceramic, and zirconia, may further substantiate this observation.[7]

The choosing of suitable restorative materials is crucial, especially when treating young children who may have issues during treatments, as choosing the right material can prevent the necessity for additional expensive and intricate interventions.[7] Composite resins and compomers are often utilized for addressing dental caries that cannot be reversed or remineralized in permanent or primary dentition. Chung et al.[29] indicated that the SR, measured using two-dimensional profilometry, was below 1 μm when the RRM surfaces seemed outwardly smooth. If the two-dimensional SR surpasses 0.2 μm, it violates the therapeutically permissible limit for RRMs.[30] In the investigation by Doğu Kaya et al.,[31] following 30 days of exposure to multivitamins, the evaluated composite did not achieve an acceptable level of SR. The resin composites submerged in the multivitamin exhibited variations in SR. Nonetheless, several resin composite samples of the current study lacked acceptable SR even at the outset which was congruent with the prior study.[30] Moreover, the resin composites within the same group exhibited varying initial SR values. This may result from the chosen region of the specimen or the presence of cavities in the material, despite adherence to conventional sample preparation protocols.[31,32]

Additionally, micro-hybrid resin composites are among the most favoured materials for direct resin restorations.[31] Additionally, nanofill composite materials incorporate submicrometer particles to augment the optical and physical characteristics of the resins.[33] Similarly, Doğu Kaya et al.[31] and Večeк et al.[34] demonstrated that the immersion of various RRMs and hybrid restorative materials in acidic beverages influenced the micromechanical characteristics, including their microhardness and SR. Both experiments demonstrated that exposure to acidic chemicals results in degradation, which varies significantly among materials based on the resin compound composition. Consequently, the choice of restorative materials is crucial for patients who frequently consume acidic beverages, as such an environment leads to the breakdown of RRMs through matrix disintegration, surface erosion, and dissolution.[35,36] This investigation revealed a substantial difference in microhardness and SR between paracetamol and hematinic syrups and the restorative materials.

Investigators have mostly concentrated on the impact of effervescent multivitamins on dental hard tissues, establishing that these medications possess erosive potential owing to their acidic composition. Nonetheless, their influence on RRM has not been sufficiently investigated. Moreover, these materials are typically the preferred choice for restorations, and various research has examined the characteristics of these materials. While restorative materials exhibit greater resistance to erosion compared to enamel, their clinical efficacy is nonetheless influenced by erosion. Acids adversely affect the physical and chemical properties of restorative materials, and their SR and microhardness.[31] Consequently, assessing microhardness and SR is crucial for comparing the efficacy of dental restoration materials, enabling professionals to make informed selections during dental treatment.[24]

The assessment of specimen surface characteristics in the present study was confined to SR and microhardness. Further research could use other criteria, including energy-dispersive X-ray examination, to evaluate the influence of pediatric syrups on the structural makeup of RRMs. The choice of restorative materials in this investigation was also constrained. Further studies may investigate the impact of pediatric syrups on a wider array of RRMs. Ultimately, the study was an in-vitro investigation, necessitating clinical trials to evaluate the impact of liquid pediatric medications on various restorative materials and enamel in practical applications.

CONCLUSION

The findings of this study indicate that increased exposure to haematinic syrup correlates with a reduction in the microhardness of RRM; a similar effect is observed in the microhardness of compomer and nanofill materials when subjected to paracetamol syrup, and also in compomer treated with antibiotic solution. The SR of the restorative material increased significantly for compomer and nanofill materials treated with haematinic syrup, in addition to for compomer exposed to paracetamol syrup.

DECLARATIONS

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

Bis-GMA: Bisphenol A-glycidyl methacrylate; TEGDMA: Triethyleneglycol dimethacrylate; TMPTMA: Trimethylopropane trimethacrylate resin;

RRM: Resin-based restorative materials; SR: Surface roughness; UDMA: Urethane dimethacrylate; VHN: Vickers hardness number.

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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SUMMARY

Low-pH pediatric syrups have been shown to cause enamel surface defects and weaken dental restorations and teeth. The study examined the microhardness and SR of three RRMs used in pediatric dentistry (compomer, microhybrid, and nanofill composite resins) after continuous exposure (at baseline, 7, 14, and 21 days) to antibiotic, analgesic, and haematinic syrups. The three pediatric syrups employed as immersion agents were amoxicillin+clavulanic acid (200 mg/28.5 mg per 5 ml), paracetamol (250 mg/5 ml), and haematinic syrup (Ferrous ascorbate (30 mg), Zinc sulphate (8 mg), Folic acid (140 mcg). The RRMs utilized were Compomer (Dyract XP), Microhybrid composite (Ivoclar Te-Econom plus), and Nanofill composite. The surface microhardness and SR were measured with a Vickers durometer and a contact profilometer respectively. The micro-hybrid materials had the highest microhardness and the compomer the highest SR among the evaluated RRMs. Contrasting the intergroup impact of the pediatric syrups on the microhardness of the RRMs showed statistically significant differences for each time, but the SR showed significant differences only at days 14 and 28.

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ORIGINAL RESEARCH

ASSESSING TUMOR MICROENVIRONMENT OF ORAL EPITHELIAL DYSPLASIA AND ORAL SQUAMOUS CELL CARCINOMA IN ORAL SUBMUCOUS FIBROSIS USING MASSON'S TRICHROME.

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ABSTRACT

Background: Oral submucous fibrosis (OSMF) is a chronic, progressive disease that affects both epithelial and connective tissue structures. Grading according to various histological parameters which include the epithelial and the connective tissue features can be demonstrated in routine hematoxylin and eosin stains(H&E). However, the use of special stains ensures better visualization which aids in early diagnosis of these connective tissue changes which includes microenvironmental changes and epithelial mesenchymal transition especially in advanced OSMF cases. The present study aims to compare the micro environmental changes and epithelial mesenchymal transition by evaluation of muscle involvement and degeneration in cases with advanced oral submucous fibrosis using Masson's trichrome

Methods: Grouping: Group 1: OED with OSMF (5 cases) Group 2: OSCC with OSMF (5 cases) Total 10 cases were taken and stained with both H&E staining and Masson's trichrome stain. This helps in assessing tumor microenvironment of oral epithelial dysplasia and oral squamous cell carcinoma in Oral submucous fibrosis using Masson's trichrome.

Results: This study shows higher amounts of inflammation in OSMF with the OSCC group, as it can be easily differentiated and helps in identifying malignant transformation. Among the two groups, oral epithelial dysplasia with Oral submucous fibrosis shows more fibrosis extension into the muscle.

Conclusion: The questionable areas of degenerating muscle bundles or areas of hyalinization were better distinguished with Masson's stain, especially in deeper connective tissue and was statistically significant. The purpose of this study is to identify potentially malignant diseases from malignant transformation of dysplasia and helps to study how collagen deposition can affect oral squamous cell carcinoma.

Keywords: Masson's trichrome, Oral submucous fibrosis, Oral epithelial dysplasia, Oral squamous cell carcinoma.

INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic, progressive disease that affects both epithelial and connective tissue structures, and is brought on by having Areca nuts in betel quid or its derivatives [1]. Chewing smokeless tobacco, eating foods high in copper, vitamin deficiencies[2], malnutrition resulting in low blood protein levels, anemia, and genetic predisposition are all considered contributing risk factors[3,4].

Different histological criteria, such as alterations in connective tissue and epithelium, are used to grade oral submucous fibrosis (OSMF). Epithelial changes like epithelial Atrophy, Epithelium thinning is frequently observed and may result in increased susceptibility to trauma[5]. Hyperplasia and Hyperkeratosis, In certain instances, there may be both hyperkeratosis (thinning of the outer keratin layer) and hyperplasia (thickening of the epithelium)[6].

Dysplasia, the existence of precancerous modifications in the cells known as dysplastic changes[7], which suggest the possibility of malignant transformation[6,8]. Then subepithelial connective tissue changes, the process of hyalinization, in which the tissue becomes glassy and homogenous as a result of excessive collagen deposition, is frequently seen in the subepithelial connective tissue. The submucosal tissues exhibit extensive fibrosis due to a substantial increase in collagen deposition. The oral tissues have become less flexible and more rigid due to an overabundance of collagen. Thick, fibrous band formation in the submucosal tissues: This condition is linked to trismus, or limited mouth opening[9,10].

In the subepithelial layer, early stages frequently exhibit a persistent inflammatory cell infiltration, primarily composed of lymphocytes and plasma cells[8]. As the fibrosis gets larger in mature stages, the inflammatory response usually decreases. Within fibrotic areas, there is a decrease in the quantity of blood vessels. Pressure from the surrounding dense collagen fibers might make the existing capillaries appear restricted or destroyed. These histological characteristics are essential for the diagnosis and treatment of OSMF because they help distinguish the disorder from other fibrotic and precancerous conditions of the oral cavity and provide light on the disease's extent[11]. Monitoring OSMF progression and probable malignant transformation requires routine biopsy and histological investigation[12].

Regular Hematoxylin and Eosin (H and E) staining could be used to identify these characteristics[13]. But in advanced OSMF cases, the application of certain stains helps to better visualize and early diagnosis of connective tissue changes like transforming fibroblasts into myofibroblasts and epithelial mesenchymal transition by providing a contrast to different components of the connective tissue[11]. We are projecting connective tissue components in this study using Masson's trichrome. Histology uses Masson's Trichrome stain, a three-color staining technique, to identify muscle, collagen, and fibrin in tissue sections[14]. For the histological assessment of disorders such as oral submucous fibrosis (OSF), it is particularly useful in analyzing fibrotic alterations[15]. The distinct hue differentiation makes it possible to see various tissue components clearly, Black-blue nuclei,cytoplasm, keratin, and muscle fibers in red color, mucin and collagen fibers in blue color[16].

Collagen fibers blue staining makes it simple to determine the degree of fibrosis [17] and to identify it, aids in comprehending the structural alterations in the mucosa and in differentiating between various tissue types, through contrasting the level of tissue fibrosis and collagen deposition across time, or before and after treatment[18]. This study compares and uses Masson's trichrome to assess muscle degeneration and participation in advanced oral submucous fibrosis.

To compare and evaluate the microenvironment of fiber and collagen deposition in Oral submucous fibrosis, sections were taken from the department block archives of patients already diagnosed with different grades of oral submucous fibrosis, patients(n=10) were grouped into two groups , group 1(n=5) Oral epithelial dysplasia with Oral submucous fibrosis(figure 1) and group 2(n=5) Oral squamous cell carcinoma with Oral submucous fibrosis (figure 2). All the ten cases were stained with both hematoxylin and eosin(H&E) staining and Masson's trichrome stain (figure 3). Staining is done with a Masson's trichrome kit. After staining all the slides were dried and are mounted with a dibutyl phthalate polystyrene xylene medium. In all the slides few parameters have been evaluated; they are fibrosis extending into muscle, vascularity, inflammation and pattern of collagen.

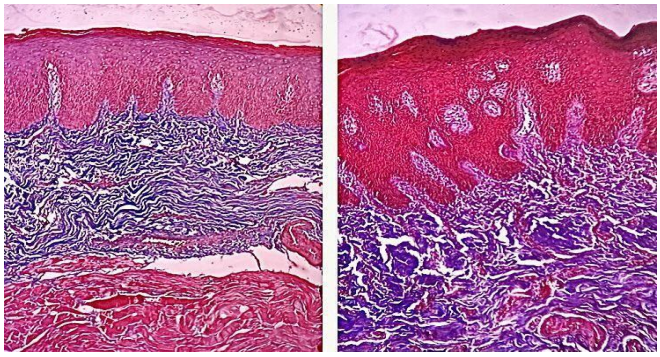


Figure 1. Masson trichrome stained sections of group 1(i.e; oral epithelial dysplasia with oral submucous fibrosis).

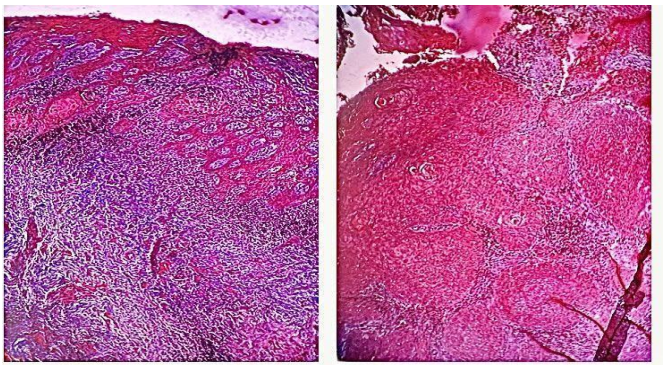


Figure 2. Masson trichrome stained sections of group 2 (i.e; oral squamous cell carcinoma with oral submucous fibrosis).

RESULT

Various parameters were evaluated and recorded like fibrosis extending into muscle, vascularity, inflammation,

pattern of collagen. Among different sets of cases, tabulation has been interpreted (table 1 and table 2). Fibrosis extension into muscle shows increased in group 2(+present, -absent). Vascularity; Shows more or less similar in both the groups (+mild,++moderate,+++intense).

Inflammation; Higher in group 2 when compared to group 1(+mild, ++moderate, +++intense). Pattern of collagen; predominantly hyalinization in group 1 while wavy in group 2(1-wavy, 2-hyalinized). Dense, widespread fibrosis with nearly total hyalinization and little inflammation were evident in the advanced stages of oral submucous fibrosis.

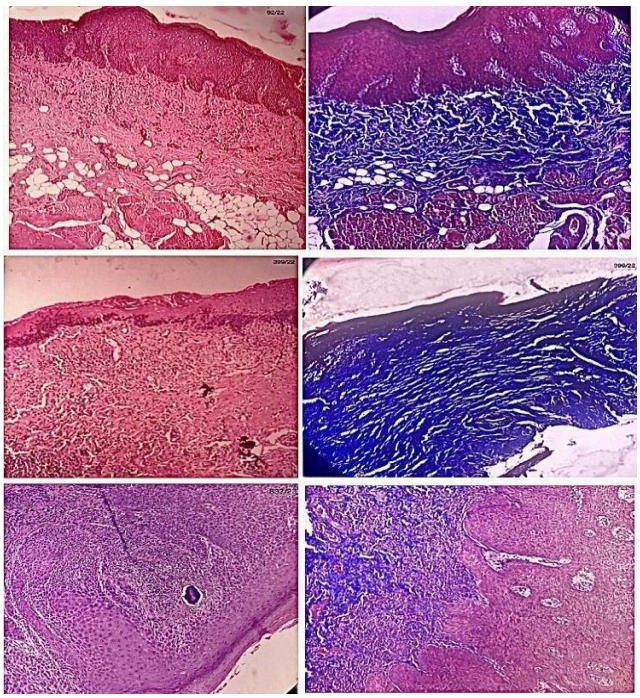


Figure 3. Both the groups stained with hematoxylin and eosin stain(on the left side) and masson's trichrome staining (on the right side)

Table 1. Table shows varying levels of collagen and fibrosis in five different patients with oral epithelial dysplasia with oral submucous fibrosis.

OED with OSMF (Group 1)	Fibrosis extending into muscle	Vascularity	Inflammation	Pattern of collagen
1	-	++	++	1
2	+	++	+	2
3	+	++	+	1
4	+	++	+	2
5	+	+	+	2

Table 2. Table shows varying levels of collagen and fibrosis in five different patients with oral squamous cell carcinoma with oral submucous fibrosis.

OSCC with OSMF (Group 2)	Fibrosis extending into muscle	Vascularity	Inflammation	Pattern of collagen
1	-	++	++	2
2	+	++	+++	2
3	-	++	+++	1
4	-	++	+++	1
5	+	++	++	2

TABLE 3. REPRESENTS THE DISTRIBUTION OF STUDY PARAMETERS IN THE STUDY GROUPS

STUDY PARAMETERS	SCORES	STUDY GROUPS	
		GROUP 1	GROUP 2
Fibrosis into muscle	0	1	3
	1	4	2
Vascularity	1	1	0
	2	4	5
Inflammation	1	4	0
	2	1	2
	3	0	3
Pattern of collagen	1	2	2
	2	3	3

Table 3 and Graph 1 presents the distribution of study parameters across two study groups. In terms of fibrosis into muscle, Group 1 had more cases with a score of 1 (4 cases) compared to Group 2 (2 cases), while Group 2 had a higher number of cases with a score of 0 (3 cases). For vascularity, most participants in both groups had a score of 2, with Group 1 having 4 cases and Group 2 having 5 cases. Regarding inflammation, Group 1 had more cases with lower scores (4 cases with score 1 and 1 case with score 2), while Group 2 had a more even distribution across scores 2 (2 cases) and 3 (3 cases). The pattern of collagen was similarly distributed across both groups, with slight variations. Overall, the distribution of parameters indicates differences in fibrosis, vascularity, and inflammation between the two groups, potentially suggesting variations in tissue characteristics or responses.

GRAPH 1 DISTRIBUTION OF STUDY PARAMETERS IN THE STUDY GROUPS

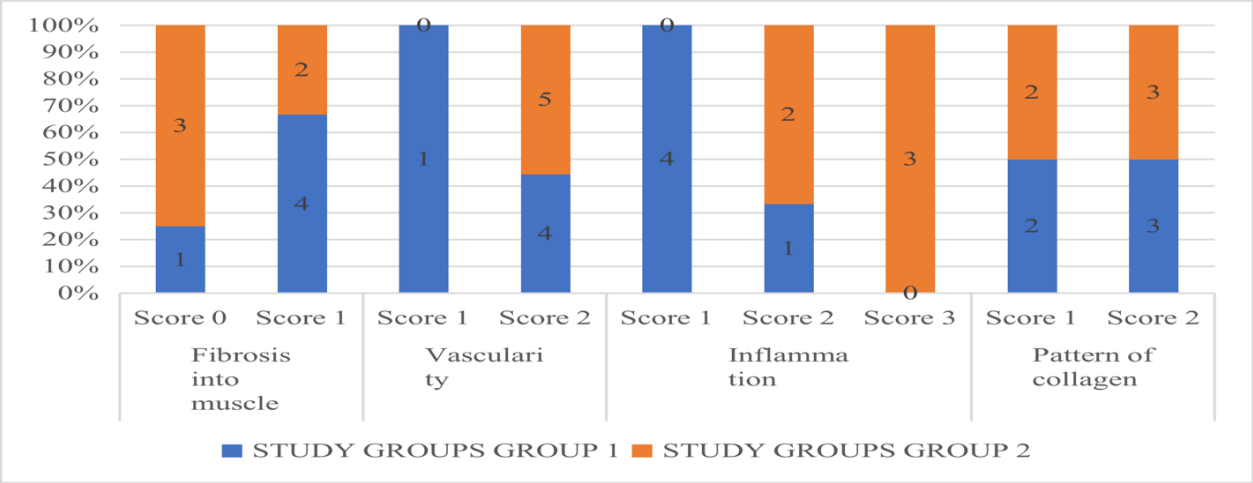


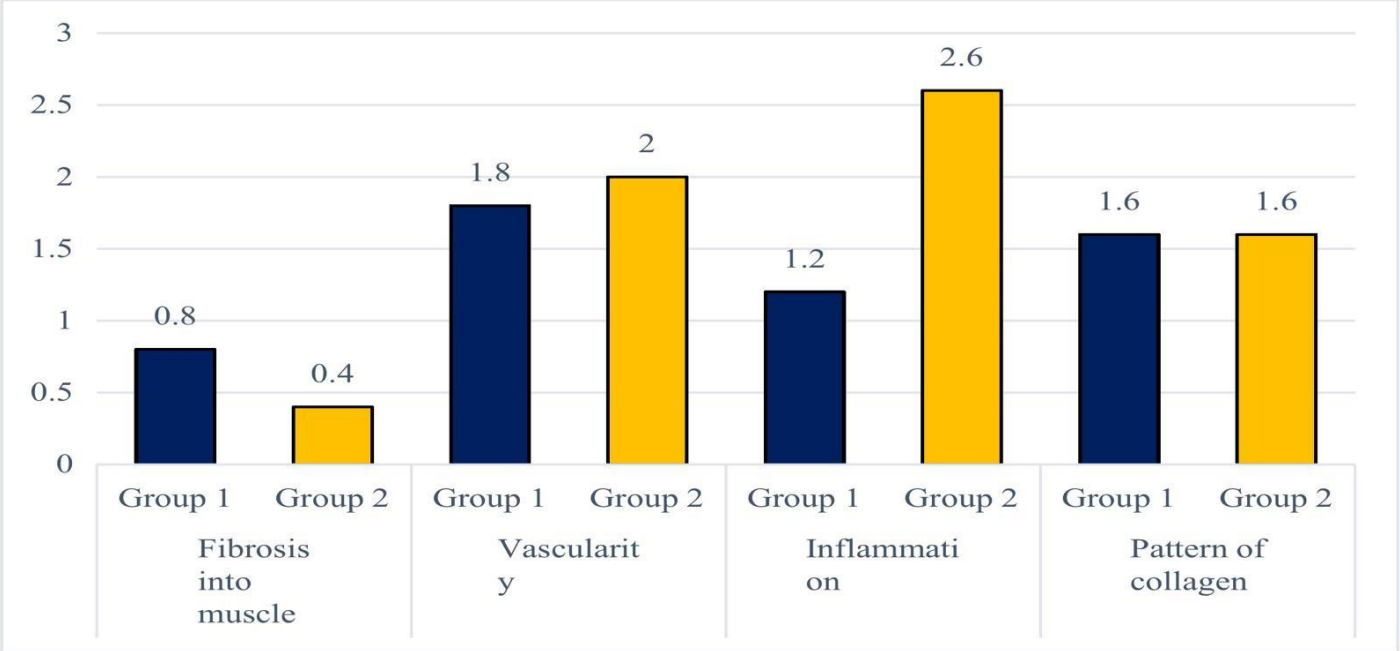
TABLE 4. REPRESENTS THE INTERGROUP COMPARISON BETWEEN STUDY GROUPS AND STUDY PARAMETERS

STUDY PARAMETERS	GROUPS	MEAN	S.D	SIG	95% CONFIDENCE INTERVAL	
					LOWER	UPPER
Fibrosis into muscle	**1**	.80	.447	.242	-.329	1.129
	2	.40	.548			
Vascularity	**1**	1.80	.447	.347	-.661	.261
	2	2.00	.000			
Inflammation	**1**	1.20	.447	.002*	-2.129	-.671
	2	2.60	.548			
Pattern of collagen	**1**	1.60	.548	1.000	-.799	.799
	2	1.60	.548			

***P value less than or equal to 0.05 is considered statistically significant different**

Table 4 and Graph 2 presents the intergroup comparison of study parameters between the two study groups. The results show no statistically significant differences in fibrosis into muscle (p = .242), vascularity (p = .347), or pattern of collagen (p = 1.000) between the groups. However, inflammation showed a statistically significant difference (p = .002), with Group 2 having a higher mean score (2.60 ± 0.548) compared to Group 1 (1.20 ± 0.447), indicating greater inflammatory response in Group 2. The confidence intervals further support these findings, particularly highlighting the significant difference in inflammation levels between the groups. Overall, while most parameters did not show significant variation, the increased inflammation in Group 2 suggests a noteworthy difference in the biological response between the groups.

GRAPH 2 REPRESENTS THE INTERGROUP MEAN COMPARISON BETWEEN STUDY GROUPS AND STUDY PARAMETERS



DISCUSSION

The chronic and severe condition known as oral submucous fibrosis (OSMF) is typified by the gradual fibrosis and inflammation of the submucosal tissues, which include the lamina propria and deeper connective tissues. It leads to noticeable stiffness and eventually the incapacity to open the mouth. The disorder is especially linked to chewing Areca nuts and is well known for its carcinogenic potential. Since the pathophysiology of OSMF is poorly understood, it is thought to be complex[1].

Possible mechanism of OSMF transforming into malignancy is illustrated by kumar bijay et al stated that Areca nut chewing will cause initiation of osmf which can be identified clinically by burning sensation (histamine) and excessive salivation (chymase) where mast cell density will increase and then release of histamine and chymase followed by release of fibroblast growth factor, vascular endothelial growth factor and transitional growth factor-ß leads to fibrosis and trismus clinically, finally malignant transformation occurs by mast cell tryptase and chymase, proliferation of myofibroblast, angiogenesis and gene mutation[19].

Another study done by kizhakkoottu et al, also stated the histopathogenesis of malignant transformation of OSMF[20]. The collagen fibers that display perivascular cuffing around blood arteries may have extravasated inflammatory cells surrounding them[8]. The surrounding collagen fiber's tendency to be hyalinized causes the blood arteries to constrict. Decreased levels of angiogenesis mediators lead to a decrease in micro vessel density as fibrosis and hyalinization advance. Clinically, these alterations manifest as mucosal blanching. Masson's stain provided a statistically significant distinction between the areas of hyalinization or degenerating muscle bundles that were dubious, particularly in deeper connective tissue. This study aims to distinguish between potentially malignant disorders and the carcinogenic transition of dysplasia and advances research on the potential impact of collagen deposition on oral squamous cell cancer[21]. Masson's Trichrome stains collagen in blue; muscle, cytoplasm and erythrocytes in red; and nuclei in blue/black color stain. This characteristic in OSMF is notably improved, making it simple to identify the dense and excessive collagen fibers that define the fibrotic bands.

Determining the degree of fibrosis can be made easier by seeing the extent of collagen deposition. The uniformity and density of the collagen are shown by the blue staining of the hyalinized areas of the subepithelial connective tissue. This characteristic is especially helpful in identifying regions with extensive fibrosis. Angiogenesis as discussed earlier plays a crucial role in tumor microenvironment which can be elevated in red color. Few inflammatory cells like mast cells, tumor associated fibroblasts can also be differentiated.

This aids in the evaluation of the tumor microenvironment in oral Submucous fibrosis for oral squamous cell carcinoma and oral epithelial dysplasia. The OSMF group in this study had higher levels of inflammation than the OSCC group, which is immediately distinguishable and aids in the detection of malignant transformation. There is greater fibrosis extension into the muscle in oral epithelial dysplasia with oral submucous fibrosis group of patients.

Pathologists find Masson's Trichrome stain to be an indispensable tool in the diagnosis of OSMF since it allows for the vivid and unmistakable distinction between different tissue types. The improved ability to see fibrotic alterations and tissue architecture aids in staining the stage of the disease by emphasizing the degree and density of fibrosis. Understanding the evolution of OSMF or the response to treatment can be aided by comparing stained sections across time. Differentiating OSMF from other oral mucosal diseases and ailments that have similar clinical presentations but different histological findings is made easier by the staining pattern's distinctive appearance.

CONCLUSION

Masson's trichrome stain was statistically significant and allowed for a better distinction between the areas of hyalinization or degenerating muscle bundles, particularly in deeper connective tissue, angiogenesis and tumor associated fibroblast and macrophages. This helps to identify epithelial mesenchymal transition. Future developments in staining techniques will even allow for the differentiation of various inflammatory cells. Differentiating cells using different stains would be recommended to facilitate further work. Aspects that affect virtual assistant deployment in dental practice. Research needs to conduct comprehensive qualitative examinations for revealing the detailed workplace attitudes of dental staff regarding virtual assistant technology adoption in their clinical work environment.

CONCLUSION

Patients together with workflow efficiency experienced significant improvement in dental practice through AI-driven virtual assistants. Thorough examination of quantitative alongside qualitative information in the study showed major enhancements affecting domains from patient satisfaction results to clinical results as well as appointment bookings and administrative performance time alongside operational workflow efficiency. The research demonstrates how AI technology will transform dental practice management to give clinicians enhanced power for delivering quality healthcare with optimized operational performance. The identification of patient satisfaction score and staff attitude predictors offers important directions for deploying strategies that yield optimal support for continuous improvement.

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"Not applicable"

Consent for publication
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Ethical approval
None

Competing interest
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CASE REPORT

MORE THAN A BUMP: DISTINGUISHING TRICHOEPITHELIOMA FROM EPIDERMOID CYST THROUGH CLINICAL AND HISTOPATHOLOGICAL INSIGHTS

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ABSTRACT

Introduction: Trichoepithelioma (TE) is a rare benign adnexal tumor of follicular origin, frequently misdiagnosed due to its clinical resemblance to other cutaneous lesions, including epidermoid cysts and basal cell carcinoma. Distinguishing these lesions is critical to prevent overtreatment or mismanagement, particularly on cosmetically sensitive facial areas.

Case Illustration: We report a case of a middle-aged female presenting with a solitary, slow-growing, skin-colored nodule on the nasal bridge. The lesion was initially suspected to be an epidermoid cyst. Dermoscopic examination revealed arborizing vessels and milia-like cysts, suggestive of TE. Histopathological evaluation confirmed the diagnosis, showing basaloid cell proliferation with follicular differentiation and keratin-filled cysts, without cellular atypia. The lesion was completely excised, and no recurrence was observed during follow-up.

Discussion: Epidermoid cysts, while also benign, exhibit different histological features such as a stratified squamous epithelial lining and keratinous content. Misdiagnosis may occur due to overlapping clinical appearance. Dermoscopy provides valuable clues, but histopathology remains the definitive diagnostic modality. Surgical excision remains the standard of care for both conditions when symptomatic or for cosmetic reasons.

Conclusion: This case highlights the diagnostic challenges in differentiating trichoepithelioma from epidermoid cysts. A multidisciplinary approach incorporating clinical examination, dermoscopy, and histopathology is essential for accurate diagnosis and appropriate management. Increased clinical awareness is crucial, especially in primary care and dermatologic settings.

Keywords*:* Dermoscopy; Differential Diagnosis; Epidermoid Cyst; Histopathology; Trichoepithelioma

INTRODUCTION

Trichoepithelioma (TE) is a benign adnexal neoplasm originating from immature hair follicles, typically appearing as solitary or multiple skin-colored papules or nodules on the face, particularly around the nose, nasolabial folds, and central facial regions. Although clinically indolent, its resemblance to basal cell carcinoma or epidermoid cysts presents a diagnostic challenge, especially in elderly patients or those with comorbidities requiring careful lesion management[1,2].

On the other hand, epidermoid cysts are among the most frequently encountered benign cutaneous cysts, developing from the proliferation of epidermal cells within the dermis. They often present as soft, slow-

growing, freely mobile nodules, occasionally with a central punctum, and may become inflamed or secondarily infected[3]. Both lesions are often confused due to overlapping clinical features, particularly when located on the face or scalp, necessitating further diagnostic evaluation. Histopathologically, TE is characterized by basaloid cell islands with peripheral palisading and keratin-filled cystic structures, whereas epidermoid cysts consist of a stratified squamous epithelium lining with laminated keratin content and absence of basaloid features[4,5]. Dermoscopy has emerged as a non-invasive diagnostic tool that may assist in differentiating these lesions based on specific vascular and pigmentation patterns[6].

Due to their similar clinical appearance, accurate diagnosis relies heavily on histopathological examination. Recent case reports and reviews highlight the importance of careful biopsy in suspected TE cases to distinguish them from malignant mimics and other benign tumors[2,5]. Given the differing management strategies, ranging from simple excision for cysts to more nuanced surgical decisions in TE, recognizing the subtle differences is critical for dermatologists and general practitioners alike.

This presents a case of facial nodule initially suspected as an epidermoid cyst but ultimately diagnosed histologically as trichoepithelioma, emphasizing the importance of biopsy in the diagnostic workup of solitary cutaneous nodules.

Case Illustration

A 56-year-old female presented with a solitary, flesh-colored nodule on the nasal region that had been gradually enlarging over the past year. The lesion was initially small, like a spot, and occasionally itchy. It was slightly tender upon palpation but non-mobile, non-ulcerated, and without signs of secondary infection. The patient denied any previous treatment or trauma at the site. Past medical history was significant for coronary artery disease, inflammatory spondylopathy, and multiple chronic medications, including methotrexate and NSAIDs. No family history of similar lesions was reported **(Figure 1).**

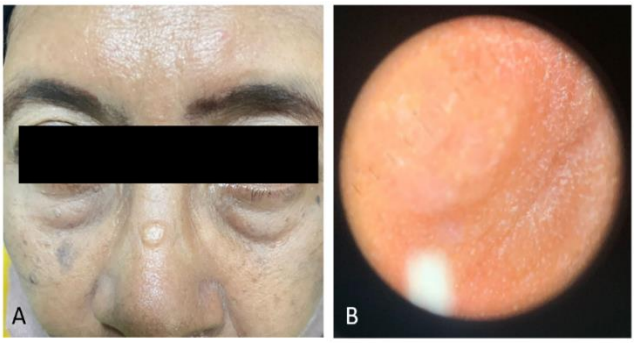


Figure 1. (A) Physical examination in first visit. (B) Dermoscopy examination shows arborizing vessels and millia-like cyst formations.

Physical examination revealed a solitaire nodul, well-demarcated, skin-colored nodule approximately 0.5 cm in diameter with no erosion. No discharge or punctum was noted. Given the clinical presentation and differential diagnosis of trichoepithelioma versus epidermoid cyst, a punch biopsy was performed. Histopathological analysis demonstrated atrophic epidermis with flat rete ridges. The dermis exhibited islands of basaloid follicular epithelial cells with peripheral palisading and the presence of keratin cysts. There were no malignant features. Based on these findings, the diagnosis of trichoepithelioma was confirmed **(Figure 2)**.

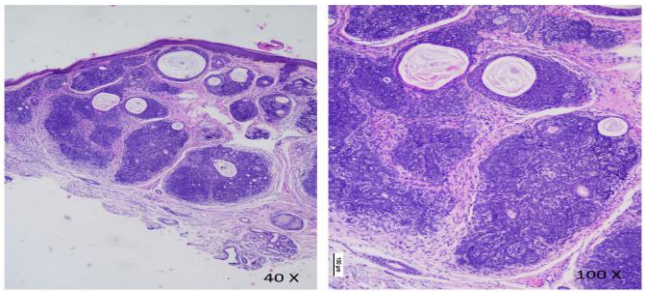


Figure 2. Shows a tissue piece coated with atrophic skin epidermis with flat rete ridge. The dermis showed proliferation of hair follicle epithelial cells forming islands with palisading edges accompanied by keratin cysts.

Therapeutic intervention involved complete excision of the lesion, intraoperative view immediately after total excision of the lesion using an elliptical excision technique under local anesthesia. Immediate postoperative view after primary closure with interrupted non-absorbable sutures, followed by oral clindamycin 300 mg three times daily and topical wound care. Gross specimen of the excised lesion sent for histopathological examination, later identified as trichoepithelioma. **(Figure 3)**. On follow up, Day 1 post-surgical appearance showing well-approximated wound edges with mild erythema and minimal edema. Day 3 follow-up demonstrating progressive reduction of local inflammation and intact sutures without signs of infection. Day 7 showing satisfactory wound healing with early signs of epithelialization and no dehiscence or hypertrophic scarring. Serial dermatological evaluations postoperatively showed good wound healing with no signs of recurrence. The patient remained stable, and cosmetic outcomes were satisfactory **(Figure 4)**.

This case highlights the importance of differentiating trichoepithelioma from other adnexal tumors such as epidermoid cysts. Despite overlapping clinical features, histopathological evaluation is essential for definitive diagnosis. Early recognition and appropriate excision prevent unnecessary concerns and ensure optimal outcomes in benign cutaneous tumors.



Figure 3. Clinical and Surgical Stages of Lesion Excision on the nose. (A) Preoperative appearance (B) Intraoperative view. (C) Immediate postoperative view. (D) Gross specimen identified as TE.



Figure 4. Postoperative Follow-Up of Surgical Site on the Nose. (A) Day 1 post-surgical appearance. (B) Day 3 follow-up. (C) Day 7 showing satisfactory wound healing.

DISSCUSION

Trichoepithelioma (TE) is benign adnexal neoplasm arising from follicular germinative cells, predominantly affecting facial regions. The clinical manifestation, especially in solitary forms, can resemble several other cutaneous tumors such as basal cell carcinoma (BCC), syringoma, or epidermoid cysts, posing a significant diagnostic challenge in routine dermatological practice[7]. In the presented case, the patient exhibited a solitary, skin-colored nodule on the nasal area that had slowly increased in size over the course of a year without ulceration, pain, or spontaneous bleeding. Such features, though typical for TE, are often underrecognized, especially in settings lacking advanced histopathological or dermoscopic diagnostics.

Differential diagnosis with epidermoid cysts is particularly challenging as both entities may present as well-demarcated nodular lesions located on the face. Epidermoid cysts, however, generally exhibit a central punctum, are compressible, and may release keratinous material upon rupture. Histologically, they consist of a stratified squamous epithelium lining and laminated keratin content, in contrast to the basaloid cell proliferation with follicular differentiation and keratin-filled cysts found in TE[8]. In the case described, the absence of punctum or discharge prompted further investigation, reinforcing the importance of not relying solely on clinical features.

Dermoscopy and histopathological analysis were pivotal in establishing a definitive diagnosis. The dermoscopic features included milia-like cysts and arborizing vessels, findings frequently associated with TE and useful in ruling out malignant conditions such as BCC[9]. Histopathological analysis confirmed the diagnosis by identifying basaloid cell islands with peripheral palisading and keratin cysts, without signs of atypia or malignancy[7,9].

Management of TE typically involves surgical excision, especially for solitary lesions with cosmetic or symptomatic concerns. The patient underwent successful excision with favorable wound healing and no signs of recurrence observed during follow-up. This aligns with existing literature suggesting excision is curative in isolated TE cases[10]. In contrast, treatment of epidermoid cysts may vary from observation to excision, particularly in inflamed or cosmetically bothersome lesions[8].

This case underscores the importance of an integrated diagnostic approach involving detailed clinical assessment, dermoscopic examination, and confirmatory histopathology to differentiate between TE and other benign or malignant adnexal tumors.

Increased awareness among clinicians regarding the overlapping clinical features is essential, particularly

in primary care or low-resource settings where access to biopsy may be limited[7,9].

In conclusion, although TE and epidermoid cysts are benign, accurate diagnosis is crucial to avoid overtreatment or misdiagnosis, especially with malignancies such as BCC. This case highlights the nuanced but clinically significant differences between these lesions and advocates for continued education and diagnostic vigilance[7,10].

CONCLUSION

Trichoepithelioma and epidermoid cyst, while both benign and often presenting as similar-appearing facial nodules, require careful clinical, dermoscopic, and histopathological evaluation to ensure accurate diagnosis. In this case, the integration of dermoscopy and histopathology confirmed trichoepithelioma and excluded other differential diagnoses such as epidermoid cyst or BCC. Prompt surgical excision yielded favorable outcomes. This case underscores the critical need for accurate diagnostic evaluation to prevent misdiagnosis and reinforces the pivotal role of histopathological examination as the definitive method for distinguishing adnexal tumors.

DECLARATIONS

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Data availability statement

The data used to support the findings of this study are included within the article.

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Consent

Written informed consent was obtained from the parent's patient for submission and publication of this case report and accompanying images.

Conflict of interest

none declared.

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REVIEW ARTICLE

USE OF TOPICAL HEMOSTATIC AGENTS IN ORAL SURGERY: MINI REVIEW

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ABSTRACT

Background: There are a huge number of hemostatic agents in dentistry that effectively prevent blood loss and heal wounds. Hemostasis with a correctly selected agent occurs instantly and reaches its peak within a few hours.
At present, new drugs affecting the hemostasis system and locally acting hemostatic agents are being intensively developed. However, despite the current situation, the issue of choosing methods for assessing the hemostatic activity of drugs in clinical practice remains open.
Material and methods: In this review, the author has summarized the literature on the use and application of topical hemostatics for bleeding in all types of oral surgery, their principles of action, and provided suggestions for selecting the right principle for different clinical situations. author conducted a literature review of scientific papers, using the resources of the Google Scholar, PubMed, Scopus, Web of, for the above keywords: Topical hemostatic agents, oral surgery, tooth extraction, bleeding, small blood loss.
Results: Local hemostatics work by activating the blood coagulation cascade at the site of vessel injury, resulting in rapid formation of a blood clot and stopping of bleeding. Efficacy depends on the mechanism of action, which can be mechanical, chemical or physiological.
Conclusion: Local hemostatics play a key role in stopping bleeding of various etiologies. These hemostatic agents provide rapid and effective cessation of bleeding, minimizing the risk of blood loss and promoting faster healing.

Keywords: Topical hemostatic agents, oral surgery, tooth extraction, bleeding, small blood loss.

INTRODUCTION

Many dental manipulations can result in damage to blood vessels. Problems related to controlling postoperative bleeding are often encountered in the daily clinical practice of dental surgeons and they use a local hemostatic agent as a means of stopping bleeding that promotes platelet activation or aggregation, forming a stable thrombus.[1-3] Tooth extraction is accompanied by bleeding of low intensity and small blood loss, since during the operation small diameter vessels (up to 100 microns) are damaged and, accordingly, low blood pressure (about 20-30 mm Hg). [4,5]The release of blood from the socket after tooth extraction is a normal physiological reaction and should be considered as a favorable outcome of the operation. The spilled blood serves as a substrate for the formation of a blood clot filling the socket, which performs both a hemostatic and plastic function (is a matrix for the tissue covering the wound defect).Patients with bleeding after tooth extraction make up from 0.25 to 5%.[6.] Bleeding that appears immediately after tooth extraction is called primary.

If it appears some time after the operation (after several hours or even days) - secondary bleeding. Alveolar bleeding is most often caused by local, less often - by general causes. Secondary bleeding is also more often associated with local factors. To stop bleeding, dentists use hemostatic agents. They are needed not only to avoid serious blood loss, but also to make it easier for a specialist to work with the patient's oral cavity. The selection of certain drugs also helps to reduce the risk of hematomas and inflammation. Hemostatic agents are indispensable tools for quick and effective stopping of bleeding, which plays an important role in the surgical procedure. To select a specific hemostatic agent for specific operations, it is necessary to have an understanding of the properties of various application hemostatic agents, their effectiveness, biodegradation characteristics, etc.

Local hemostatic agents Characterized by targeted action and used in case of damage to large vessels and diffuse bleeding. Often used in emergency surgery. Stop capillary and parenchymatous bleeding.[7-9]

Local hemostatic agents have a wide range of effects on the affected area and are selected based on a specific situation. They are aimed at preventing blood loss. Mechanical, thermal and chemical local hemostatic agents are distinguished. Requirements for hemostatic drugs in dentistry.[10,11]

Mechanical: Mechanical means intended to stop bleeding in the oral cavity include coagulating components. They create a physical barrier to bleeding, allowing the blood to quickly clot and form a clot. They are selected based on the nature of the bleeding, the structural features of the jaw, the condition of the gums and other nuances that the attending physician takes into account during the manipulations.For tamponade of the socket in case of post-extraction bleeding, gauze soaked in thrombin, epsilon-aminocaproic acid, hemophobin, amifer, as well as a hemostatic sponge or gauze, a fibrin sponge (film, cotton wool, foam), fibrin glue, a biological antiseptic tampon (BAT), a gelatin or collagen sponge and other means can be used.[12 -15]

Thermal: These are special devices for coagulation, the operation of which is based on physical principles. This includes electrocoagulation, photocoagulation, laser, ultrasound, radiofrequency, microwave, argon plasma coagulation.

Chemical: Local chemical hemostatic agents include various applications that directly affect the blood coagulation system.[16,17] The ideal hemostatic agent should be biocompatible, easy to apply, safe and have an immediate effect. Local hemostatic agents can be classified as: [18]

1. Passive hemostatic agents

- Collagen-based products:Microfibillar collagen (Avitene) Absorbable collagen hemostat sponge (Helistat) Colla-Cote, Colla-Tape, Colla-Plug.
- Cellulose-based products; Oxidized regenerated cellulose (Surgicel) ActCel and Gelitacel
- Gelatin-based products: Gelfoam
- Polysaccharide hemospheres

Passive hemostatic agents form a physical matrix that adheres to the bleeding site and provides the core a platform around which platelets can gather unite, forming a blood clot. They are suitable for use as a first line agents, because they are easily accessible, not special storage is required and relatively cheap.

2. Active hemostatic agents

- Thrombin
- FloSeal (flowablehemostatic agent)
- Sealants Fibrin sealant (tisseel)
- Albuminderived hemostat(bioglue)

Topical Thrombin: Thrombin is used as an effective means of stopping bleeding by converting soluble fibrinogen to insoluble fibrin, forming a fibrin clot. It includes bovine thrombin (Trombin-JMI), human plasma-derived thrombin (Evithrom), and recombinant human thrombin (Recothrom). Human thrombin offers efficacy and less immunological response than bovine thrombin. Hemoblast™ contains three components: human thrombin, collagen, and chondroitin sulfate[19].

FloSeal (flowablehemostatic agent);Thse materials are a mixture of a flowing gelatin matrix (porcine or porcine) and a human-derived thrombin component that facilitates fibrin formation, promoting clotting and minimizing blood loss. Floseal® is composed of a bovine gelatin matrix and a porcine gelatin matrix and is indicated in surgical procedures as an adjunct to hemostasis[20,21].

Fibrin sealant (tisseel): Fibrin sealants contain fibrinogen and thrombin, where thrombin converts fibrinogen to fibrin and activates factor XIII. There are currently many different fibrin sealant products available, including dry fibrin TachoSil, Tacho-Comb, fibrin pad, dry fibrin sealant dressing, and Fibriseal[22,23]. Liquid fibrin sealants come in two separate vials in which fibrinogen and thrombin are mixed immediately before use. Tisseel, Crosseal, Evicel, and Quixil are examples of liquid fibrin sealant products.

Kaprofer:Pharmacological action (pharmacodynamic) Pharmacological action - hemostatic.[24,25] The combined drug has a local hemostatic effect that occurs immediately after its application. When interacting with blood, a blood clot is formed, firmly fixed on the wound surface, prevents repeated bleeding and prevents the penetration of secondary infection. Accelerates regeneration and postoperative epithelization of the wound surface, formation of granulation tissue. It also has anti-edematous and anti-inflammatory effects. Pharmacokinetics Indications for use Bleeding (in various dental procedures, in the treatment of pulpitis, to stop bleeding from tooth canals after tooth pulp extirpation), in surgical interventions on periodontal tissues to stop bleeding and remove collateral swelling of soft tissues in the postoperative period. Method of application and dosage With persistent bleeding, a cotton swab can be left in the hole for a day. The tampon is removed independently. In the canals of the teeth and gingival pockets, the drug is injected for 2-5 seconds.

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CASE REPORT

RANULA:A CASE STUDY

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ABSTRACT

Ranulas form harmless mucous cysts under the tongue area because of blocked or damaged sublingual salivary gland tubes. The cysts produce problems with movement and feeling which need medical treatment.

This medical illustration shows how doctors treated and healed simple ranulas in a patient through surgery. The doctors diagnosed and removed a problem ranula through surgery from a male patient. During treatment we protected important tissues while removing the simple ranula and the patient recovered quickly without major problems. The operated area shows excellent healing without any signs of infection and the patient will fully heal.

Keywords: Ranula, mucous cyst, sublingual salivary gland, cyst removal, oral surgery

INTRODUCTION

A ranula is a type of mucous cyst that forms in the floor of the mouth, typically resulting from the blockage or rupture of a salivary gland duct, leading to the accumulation of mucus. Exterior cystic formations of ranulas appear in two distinct ranges: tiny clear blotches and noticeably bigger shapes which interfere with communication and produce swallowing and breathing restrictions [1]. Medical intervention becomes necessary for ranulas to resolve when such cysts generate functional problems with oral functions or when they persist. The medical term "ranula" comes from Latin roots referring to the condition because this formation resembles the extended stomach of a frog. Ranulas develop mainly within sublingual salivary glands, yet they can appear in either submandibular or parotid salivary tissue [2]. The occurrence of these cysts remains more frequent in adults, while children can develop them too, and their appearance is most typical between the ages 20-40 years. Research shows that the cause of ranulas includes ductal system blockage or traumatic events, but some cases have no identifiable factor [3].

Simple ranulas represent one type of the condition, while plunging ranulas form the other classification. The simplest form of ranulas develops in the floor of the mouth and tends to stay small in size. The swellings of ranulas present as clear or bluish or translucent, and they are often painless or only mildly tender. A plunging ranula advances from the floor of the mouth into the neck so it resulting in enlarged neck tissue which becomes more noticeable [4]. Treatment and diagnosis of plunging ranulas prove highly challenging since these lesions extend deeply while involving important structures near the mouth. The cause of ranulas typically includes ductal obstruction or rupture within the sublingual salivary gland, but any salivary gland can develop a ranula [5]. Ductal obstruction takes place mainly because of trauma and ductal stenosis, or mucus plug formation.

Ranulas develop under extremely rare circumstances from both congenital defects and developmental abnormalities within salivary glands. The risk of developing ranulas has been demonstrated to rise because of dehydration, together with reduced salivary flow [6]. Patients usually experience painless or slightly painful floor-of-mouth swelling while examining a ranula during their visit. The large size of ranulas, together with their mouth location, produces discomfort that becomes most noticeable during eating and speaking, and swallowing. Symptoms of tongue movement limitation lead to speech problems along with difficulties in chewing because of the swelling [7]. The infection of a ranula produces pain that accompanies inflammatory indications, including skin redness, together with pus-tainted fluid. Medical identification of a ranula requires a complete clinical assessment since healthcare providers must distinguish the cyst from both tumors and other oral pathologies, such as cysts [8]. Medical diagnosis of ranulas begins with clinical observations that healthcare providers can confirm using imaging methods such as ultrasound and sialography, as well as magnetic resonance imaging. Visual imaging modalities show the dimensions and exact placement and deep reach extent of the cyst, especially when plunging ranulas spread from oral tissues into neck structures. The diagnostic tool of choice for doctors is generally ultrasound because it delivers cost-efficient, non-invasive examination of soft tissue masses [9].

Case presentation

A male patient aged 25 years presented with a complaint of a persistent swelling in the floor of the mouth that had been progressively increasing in size since 3 months. The patient felt mild pain only while speaking and swallowing although there was no severe pain or infection detected. The healthcare professional performed an examination that revealed the tongue-shaped tumorous mass to be a translucent smooth lesion under the tongue. A ranula could be diagnosed from intraoral examination because the floor of the mouth contained a visible swelling. The patient's ranula had a translucent appearance with dome shape dimensions that measured about 1 to 1.5 cm within the sublingual region. The cyst presented feel smooth when touched and displayed both non-painful and spongy qualities that help identify this cyst as a mucous retention cyst. The small swelling partially shifted the tongue in position thus creating potential difficulties during speech and mastication functions. At this point the area displayed no evidence of infection together with the absence of erythema.

Differential Diagnosis: The clinical appearance of the lesion suggested a diagnosis of ranula, a mucous cyst commonly associated with the obstruction or rupture of the sublingual salivary glands. However, other potential diagnoses considered included:

- **Sublingual abscess** – less likely due to the absence of acute pain, fever, or purulent discharge.
- **Dermoid cyst** – could present similarly but is typically firmer and less fluctuant.
- **Lipoma** – often presents as a more solid, yellowish mass, less likely to be translucent.
- **Other types of oral cysts or tumors** – requiring confirmation via imaging.

The patient was referred for further diagnostic imaging to confirm the diagnosis and assess the extent of the cyst. An ultrasound of the lesion revealed a fluid-filled, well-circumscribed mass consistent with a ranula. MRI or sialography may be considered for deeper involvement or if there is suspicion of a plunging ranula extending into the neck.



Figure 1. Pre-operative (clinical appearance of the lesion suggested a diagnosis of ranula)

Intraoperatively, the cyst was carefully removed after making a small incision, with minimal disruption to surrounding structures. The surgical area received closure through non-absorbable stitches which enabled proper wound healing while minimizing recurrence possibilities. The patient received treatment for pain while doctors provided antibiotics together with advice about proper oral hygiene practice. The healthcare providers arranged subsequent appointments after surgery to track the healing progress as well as check for infection development and recurrence potential while teaching the patient about potential risks alongside expected outcomes.

The surgical site appears to be healing well with minimal swelling or erythema. There is no visible active bleeding, and the mucosal tissue is intact, with signs of early healing. The skin shows slight redness because tissue healing is natural during early recovery periods. The patient's recovery will be assessed during

subsequent visits for both infection signs and complications along with recurrence. The healing process will unfold smoothly when patients follow oral hygiene practices along with any provided medications. Additional visits to the healthcare provider will be necessary for cyst resolution confirmation as well as verification that all postoperative problems have disappeared.



Figure 2. Intra operative (cyst was carefully removed after making a small incision)



Figure 2. Intra operative(surgical area received closure through non-absorbable stitches)



Figure 3. Post-operative (floor of the mouth after the excision of the ranula)

DISCUSSION

Ranulas are a common type of mucous cyst that forms in the floor of the mouth, typically resulting from the obstruction or rupture of the duct of the sublingual salivary gland. They are often characterized by a soft, fluctuating swelling beneath the tongue that can cause discomfort, especially when speaking or eating.

The general benign nature of ranulas requires medical treatment because their specific positions and sizes can disturb mouth function. Single and plunging ranulas represent the two primary subdivisions of this condition. Simple ranulas stay within the oral cavity to display small transparent swellings, yet plunging ranulas penetrate the floor of the mouth to reach neck tissues as well [10]. The diagnosed ranula presented as a simple form, which remained within the floor of the mouth, thereby enabling easier surgical intervention. The course of therapy for ranulas depends on cyst dimension, along with patient symptom severity and relapse probabilities. The medical procedure of surgical removal stands as the most common therapeutic approach when dealing with both big or causing symptoms of ranulas. Small to recurrent lesions typically require the surgical technique of Marsupialization instead of excision [11]. The main purpose of surgery includes complete cyst removal while ensuring no harm comes to the submandibular ducts and lingual nerves to prevent postoperative complications. The presented treatment included ranula excision along with preservation techniques for surrounding tissues. Favorable surgical outcomes become apparent in the postoperative image because the tissue shows minimal swelling along with no indications of infection, thereby indicating that surgical steps were successful. The development of minimal redness at the surgery site is a typical healing response which should disappear when the tissue restores [12].

CONCLUSION

It is concluded that ranulas are benign mucous cysts that can be effectively managed with surgical intervention, particularly when they cause discomfort or interfere with normal oral functions. In this case, the surgical excision of the ranula was successful, with favorable postoperative healing and minimal complications. The careful removal of the cyst while preserving surrounding structures ensured a smooth recovery, and the patient is expected to experience a full resolution of symptoms. Early diagnosis, appropriate surgical treatment, and diligent postoperative care are key to preventing recurrence and ensuring optimal outcomes.

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Competing interest

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CASE REPORT

RESTORING FUNCTION AND QUALITY OF LIFE: A CASE REPORT OF FIXED FUNCTIONAL GUIDE FLANGE PROSTHESIS IN HEMI MANDIBULECTOMY REHABILITATION

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ABSTRACT

Hemi mandibulectomy, as a surgical removal of one lateral half of the mandible, tends to result in patients with considerable esthetic and functional deficits such as mandibular deviation, masticatory disability, speech defect, and facial asymmetry. Prosthetic rehabilitation is required to restore function and quality of life for such individuals. The present case report illustrates prosthodontic rehabilitation of a 65-year-old man who had undergone right hemi mandibulectomy. A fixed guide flange prosthesis was designed and made for mandibular guidance and prevention of deviation on closure. The prosthesis harnessed the available dentition to stabilize it and provided a mechanical guide plane for optimal occlusion. The patient exhibited significant improvement in masticatory efficiency, mandibular control, and overall oral function. This case demonstrates the effectiveness of a fixed guide flange prosthesis in rehabilitation following post-hemi mandibulectomy and emphasizes the significance of early prosthodontic intervention.

INTRODUCTION

Oral cancer is the sixth most prevalent form of cancer worldwide. India bears the highest number of oral cancer incidences, accounting for one-third of the total global burden of this disease.[1,2.] Buccal mucosa cancer typically manifests along the occlusal plane and is distinguished by pain and ulceration, often accompanied by a buccal mass. Squamous cell carcinoma (SCC) affecting the buccal mucosa is infrequent, constituting approximately 10% of all oral cancers.[3,4.] It is widely accepted that patients with oral squamous cell carcinoma involving the mandible should undergo surgical treatment. In cases of significant mandibular invasion, a mandibular resection

is necessary.[5]

Following mandibulectomy, patients typically experience the loss of occlusion and the absence of muscle attachment to the mandible on the surgical side. This leads to inferior rotation of the mandible upon closure, causing deviation towards the side of the defect. Additionally, it results in facial deformity and difficulties in speech and mastication.[6] The treatment of a deviated mandible typically begins with early corrective mandibular movement therapy, which involves physiotherapeutic stretching exercises. Additionally, different designs of prostheses have been described to aid in guiding the mandible into centric occlusion.[7,8,9.] The literature describes a variety of methods for correcting mandibular deviation, including intermaxillary or palatal fixation with elastics or mandibular guiding flange prosthesis fixed to the natural teeth or dental flange. A removable guide flange prosthesis is difficult to be retained intraorally if a few teeth remain in the segmented mandible. Radiation and surgical scarring can further impair retention by limiting mouth opening and functional vestibule depth, making it impossible for the patient to secure and remove the guide flange prosthesis and causing more occlusal issues.[10] There are several approaches to rehabilitate a patient with a permanent or removable guide flange prosthesis.[11-16.]

This article aims to provide patients with oral carcinomas a prosthesis that is functional, affordable, rigid enough to resist masticatory forces, for patients who belong primarily from families with low incomes, for a disease which is a result of smoking and ingesting toxins. This case report describes a fixed dental guide flange prosthesis, a financially viable choice for individuals from lower socioeconomic strata, where reconstruction procedures and endosteal dental implants are not affordable.

CASE PRESENTATION:

A 65-year-old male patient presented to the Department of Prosthodontics with a chief complaint of difficulty in chewing meals. Upon recording the patient's complete medical history, it was revealed that he had experienced pain in his right jaw and teeth region six months prior. Subsequent diagnosis indicated carcinoma of the buccal mucosa, leading him to undergo hemi mandibulectomy along with resection of the right lateral border of the tongue, which was replaced with a PMMC flap. Due to post-surgical challenges in mastication and speech, the patient was referred for prosthetic rehabilitation.

During the extra-oral examination, a limited mouth opening of 20mm was noted, along with mandibular deviation to the affected side, resulting in facial asymmetry (Figure 1). Intraoral examination and OPG (Figure 2), revealed missing teeth with 15, 16 and 36, with root stumps remaining for 14 and 17. While considering various treatment options, including implants, the possibility of an implant-supported fixed prosthesis was ruled out due to the patient's history of radiation therapy. Given the absence of tooth 36 and the patient's preference for its replacement, it was decided to fabricate a fixed functional guide flange prosthesis, utilizing 35 and 37 as abutments.



Figure 1. Mandibular deviation of the affected side.



Figure 2. OPG

CLINICAL PROCEDURE:

- Impressions of maxillary and mandibular arches were made using irreversible hydrocolloid (Zhermac Tropicalgin) and poured with type III gypsum (dental stone) material. Deviated bite was recorded using jet bite material and mounted on a mean value articulator.
- Tooth preparation for receiving a metal FPD on the missing 36 tooth was performed followed by secondary impression recording after gingival tissue retraction using a 000 gingival retraction cord and two-stage putty light-body impression with addition silicone. Mandibular cast was poured using type IV gypsum (die stone) material. (Figure 3)
- Provisionalization of prepared teeth was carried out using Protemp 4 (3M ESPE).
- Facebow transfer and centric bite recording was completed using silicone material (Jet bite, Coltene), and the casts were mounted on a semi-adjustable articulator.

- The wax pattern was fabricated, and for the extension of the guide flange, 2mm sprue wax was bent at right angles, resembling a U-shape, from the buccal aspect of the abutment teeth wax
  pattern (Figure 4). This arrangement provided support for the clear acrylic resin. Self-cure clear acrylic material was adapted to the metal extension, extending from the buccal aspect, ensuring that there was no impingement on the maxillary gingiva during maximum intercuspation.
- Trial was conducted to assess mandibular guidance with the guide flange prosthesis, with the patient exhibiting ease in guiding the mandible into the centric position without discomfort.
- The functional prosthesis was cemented using type 1 Glass ionomer cement. (Figure 5- A and B). After cementation, the patient was given appointment for follow-up visit.



Figure 3. Maxillary and mandibular definitive casts.

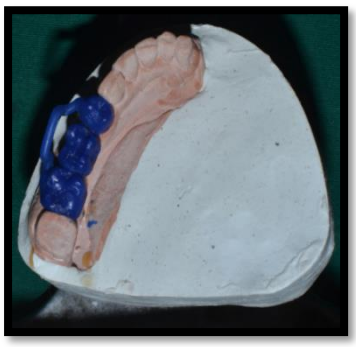


Figure 4. Wax pattern of the guide flange prosthesis.

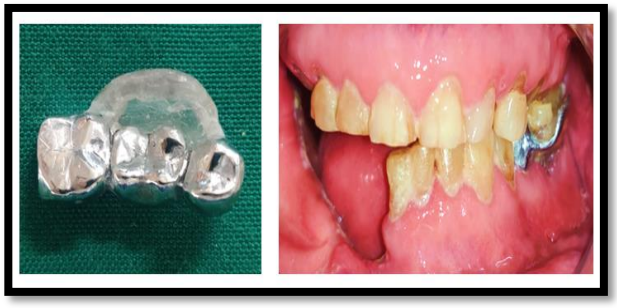


Figure 5. A. Final guide flange prosthesis with clear acrylic extension. B. Cementation of the prosthesis intraorally.

FOLLOW-UP:
The recall visit was scheduled 24 hours post the prosthesis cementation. Any issues arising from acrylic extension impingement were promptly addressed and rectified to ensure patient comfort. Notably, as the prosthesis was fixed, the inconvenience of removing and replacing the prosthesis was not an issue, addressing a significant limitation of removable prostheses. Subsequent to the initial visit, the patient was re-evaluated within a month's timeframe.

During this follow-up, the patient expressed satisfaction with improved mandibular closure facilitated by the fixed prosthesis, enhancing the patient's ability to masticate, improved phonetics, and reported an overall sense of psychological well-being.



Figure 6. Pre-operative photograph depicting mandibular deviation. B. Post-operative photograph with prosthesis in-situ.

DISCUSSION

Following oral cancer surgery, an undesirable anatomic and biomechanical state is frequently created, such as limited mouth opening due to scarring, reduced tongue function, loss of labiolingual sulcus, mandibular deviation, absence of underlying osseous support for facial characteristics, and insufficient soft tissue for speech and swallowing.[17]
Rehabilitating mandibular abnormalities is essential to regain oral function.[18] Therefore, the primary goal of this case report was to propose a fixed prosthesis for the patient, which would help with the process of restoring oral function.
This article discussed a case study of a patient who was diagnosed with squamous cell carcinoma of the buccal mucosa for which he underwent right hemi-mandibulectomy and had the lateral border of the tongue resected, then a PMMC flap used for the reconstruction. The patient had a history of chewing beetle nut for 20 years and had poor maintenance of oral hygiene.
To undergo prosthodontic rehabilitation, it is essential to comprehend the various classifications of mandibular defects given by authors like Jewer and Boyd, Urken,

Cantor and Curtis. [19-22] This article deals with Cantor and Curtis class II type of a mandibular defect, for which a fixed functional guide flange prosthesis in the form of a metal FPD was planned. There are different methods of rehabilitation using fixed or removable prosthesis for such defects. In some cases, scar contraction limits the mouth opening in a more extensive manner that the placement and removal of removable appliances become difficult. Therefore, a fixed guide flange prosthesis is designed in such cases, which would keep the muscles in a stressed condition, preventing scar contraction[13].

Nelogi et al. developed a fixed guide flange prosthesis by affixing an orthodontic band around a molar tooth. They then adapted a wrought wire into a U shape, with a tube 10mm in length positioned at the base of the U-shaped framework. This allowed for free rotation around the tube, facilitating the guidance of the mandible into centric position.[9] Since there was a possibility of an FPD owing to missing 36, it was decided to fabricate a buccal extension as a guide onto the metal FPD which would guide the mandible into centric. In this prosthesis, a wax pattern was fabricated onto 35-37 with a U-shaped extension onto the prepared wax pattern using sprue wax which extended buccally onto the maxillary molar. After fabrication of the metal FPD, clear acrylic was adapted onto the metal extension to act as a guide and was extended until the maxillary molar. This technique is suggested exclusively when the remaining teeth exhibit sufficient periodontal health to withstand the masticatory forces and muscular pull.

The proposed fixed guide flange prosthesis is not only functional but also easy to fabricate and repair, comfortable and esthetic. Moreover, it facilitates improved hygiene maintenance.

Following the cementation of the prosthesis, it is important to conduct regular evaluations of the patient. Initial assessments should occur at intervals of 24 hours and 72 hours to assess for muscle pain, discomfort around the temporomandibular joint (TMJ), or any potential impingement of the acrylic extension onto the maxillary buccal mucosa. Subsequently, evaluations should be conducted at monthly intervals to gauge the effectiveness of the guide flange prosthesis.

CONCLUSION

The suggested fixed guide flange prosthesis serves as an alternative to removable guide flange prostheses which is easy to fabricate and repair while improving the ability to masticate, enhance support, as forces are distributed along healthy abutment teeth. Along with these benefits, the prosthesis is cost-effective and beneficial for patients who belong to lower socio-economic status. Long-term studies are warranted to comprehensively evaluate both the effectiveness and potential drawbacks of employing fixed guide flange prostheses as a prosthetic rehabilitation option for hemimandibulectomy defects.

DECLARATIONS

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Competing interests

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ORIGINAL RESEARCH

CORRELATION BETWEEN FAGERSTRÖM TEST FOR NICOTINE DEPENDENCE AND PREVALENCE OF ORAL MUCOSAL LESIONS: A CROSS-SECTIONAL STUDY

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ABSTRACT

Tobacco use is a major risk factor for oral mucosal lesions (OMLs), including potentially malignant disorders. The Fagerstrom Test for Nicotine Dependence (FTND) is a tool for assessing nicotine dependence. This study investigates the potential correlation between FTND scores and the prevalence of OMLs. The present study aims to determine if a correlation exists between FTND scores, indicating nicotine dependence levels, and the prevalence of Oral mucosal lesions. Patient records from a Dental Information Archiving System Software were analyzed to identify individuals with tobacco use habits. FTND scores were calculated to assess nicotine dependence levels, and oral mucosal changes were recorded. Male patients aged 18 years above with a history of cigarette smoking and tobacco usage, 70 participants in each group were included. The mean age of the patients was 37.6 +/- 13.2 years with an age range of 18 to 75 years. Individuals with more severe oral mucosal lesions tend to have higher scores on the Fagerstrom Test for Nicotine Dependence, suggesting a stronger nicotine addiction. A weak, positive correlation between Age and Mucosal Changes was observed (r = 0.089). A moderate, negative correlation between Mucosal Changes and Tobacco Cessation Form (r = -0.279). This correlation was statistically significant at the 0.01 level (p = 0.001). The present study highlights a link between nicotine dependence and oral lesions, emphasizing the need for targeted intervention toward habit cessation.

Keywords: Fagerstrom test, nicotine dependence, oral mucosal lesions, prevalence,

INTRODUCTION

Tobacco, originating from the *Nicotiana* genus in the Nightshade family, has been used since the first century BC by the Maya tribe in Central America for religious ceremonies, and its consumption spread across South Asia in various forms such as hookah, cigarettes, and smokeless tobacco [1,2]. Cultural influences and genetic predispositions continue to shape tobacco use, with habits such as gutkha consumption providing users with benefits like focus and relaxation [3, 4].

India's tobacco industry is a major economic contributor, employing millions and generating substantial foreign exchange. As the world's second-largest producer and fourth-largest exporter of unmanufactured tobacco, India offers a diverse range of products to over 125 countries. While the industry provides economic benefits, it also faces challenges related to labor conditions, particularly in bidi manufacturing, and environmental concerns. Government initiatives are essential to promote alternative livelihoods and reduce dependence on tobacco cultivation and production [5, 6, 7].

The primary biohazardous substances found in tobacco include at least eighty-three carcinogens, including nitrosamines and nicotine, and radionuclides that produce alpha particles, such as polonium 210 [8]. Tobacco smoke is packed with chemicals that harm the body's defenses against disease [9], including tars, thiocyanates, herbicides, fungicides, and pesticide residues, as well as carbon monoxide. Tobacco smoke contains toxic chemicals which adversely affect almost all types of living cells. First and foremost, smoking has a detrimental effect on the host's immune system, which in turn affects how the host and parasite interact. In addition to its obvious negative health effects tobacco use, whether inhaled, sniffed, sucked, or chewed has an addictive quality. Researchers have conclusively shown that tobacco users experience the three Ds: disease, disability, and death [10].

When any cell is exposed to a carcinogen of any kind it often strives to adapt to it. A possible adaptation strategy could be a rise in cell division, a decrease in cytosolic capacity, and an increase in the load of related organelles [11, 12]. The early consequences of an accelerated growth phase in the oral epithelium are indicated by hyperplasia or enlargement of the progenitor compartment. The epithelium exhibits characteristics of cellular degeneration, a well-known adaptive feature (atrophy) when the irritant persists. The cells eventually progress to an irreversible cell damage stage, which can take the form of either apoptosis or malignant transformation, after the stages of adaptation and reversible cell damage are over. The accelerated rate of cell division seen in the early phases of transformation is an adaptive response that promotes more genetic damage and drives the cells farther down the path toward malignant transformation [13].

The Fagerström Test for Nicotine Dependence(FTND) is a widely used tool to assess the level of physical addiction to nicotine. Apart from FTND other scales such as the Heavy smoking index (2-item scale), and cigarette dependence scale(5-item scale) are also used however FTND remains the gold standard [14]. FTND scale is designed to provide a graded measure of nicotine dependence related to cigarette smoking, the test includes six questions that evaluate cigarette consumption, the urge to smoke, and overall dependence. A higher total score on the Fagerström test indicates a greater physical dependence on nicotine. A doctor may use the Fagerström test in the clinic to record when a patient needs a prescription for medicine to aid with nicotine withdrawal. Karl-Olov Fagerström originally developed the Fagerström Tolerance Questionnaire. In 1991, Todd Heatherton and colleagues modified this questionnaire, resulting in the Fagerström Test for Nicotine Dependence[15, 16]. This scale is also used to determine the level of nicotine dependency based on the form of tobacco used (ie) smoking, or chewing. The present study aims to determine the relationship between the Nicotine dependency score and the corresponding mucosal changes observed concerning the patient's habit.

Methodology

A retrospective study was carried out in the Department of Oral Pathology at Saveetha Dental College, Chennai. This retrospective design utilized stored patient records to identify patterns and associations. The data for this study was sourced from the Dental Information Archiving Software (DIAS). This digital database stores patient information, including dental records and treatment histories at Saveetha Dental College and Hospitals. Patients with tobacco habits reporting to the dental OP for routine treatment were assessed for Nicotine dependence based on the Fagestorm score for nicotine dependency. Further information on the mucosal changes observed in the patient was also recorded. The study was conducted under the Declaration of Helsinki, and ethical clearance was obtained. **(IHEC/SDC/DS/FACULTY/23/OPATH/141)**

Population: The study focused on individuals who reported to a dental clinic for regular treatment and had a history of tobacco use, including both smoking and smokeless forms.

Sample Size: Sample size was calculated using the odds ratio value obtained from a reference article [17] using G power software v 3.1.9.4. It was calculated to be 78 samples with 39 samples in each group. A total of 500 patients were screened for the study of which only male patients were included in the study. The final sample size included 140 patients including 70 tobacco smokers(cigarettes) and 70 tobacco chewers to eliminate the risk of bias among both forms of tobacco usage. Inclusion criteria were, Male patients above the age of 18 years with a history of tobacco usage. In the smoking category, only cigarette smokers were included to maintain homogeneity. Case records were excluded if they lacked sufficient information due to missing scores and incomplete forms. Participants who smoked beedi and other smoke forms of tobacco were excluded due to insufficient sample size. Female participants were excluded due to insufficient sample size in both categories compared to male participants. Male smokers were chosen to ensure homogeneity in the study population and to minimize gender-based variations in physiological and behavioral responses. Participants using both forms of tobacco were excluded to maintain consistency in the categorization.

Data Collection:

Patient Selection: Existing patient records were reviewed to identify individuals who met the inclusion criteria (tobacco use, and regular dental visits). Data Extraction: Relevant information, such as patient demographics, tobacco use history, frequency of smoking and tobacco chewing, details of oral mucosal changes, and Fagerstrom score were noted, were extracted from the DIAS database. The data obtained were entered into Microsoft Excel version 2022 and statistical analysis was done using SPSS software version 26.

The FTND questionnaires were used by the clinicians to assess the level of nicotine dependence through a one-on-one interview with the patient. Fagerstrom Test for Nicotine Dependence consisted of yes/no items which were scored from 0 to 1 and multiple-choice items scored from 0 to 3. The items are summed to yield a total score of 0-10. Scores are categorized as very low (0–2), low (3–4), medium (5), high (6–7), and very high dependence (8 or more). The higher the total Fagerström score, the more intense is the patient's physical dependence on nicotine [15,16]. This questionnaire was then assessed by a senior clinician following which the questionnaire was scanned and uploaded into the dias software with prior informed consent from the patient.

Data Analysis:

Descriptive Statistics: Summary statistics were calculated to describe the characteristics of the study population, including age, gender, duration of tobacco use, and type of tobacco products.

Statistical tests - Pearson's correlation was employed to examine the relationship between the frequency or severity of tobacco use (as measured by the Fagerstrom Score) and the presence of mucosal changes in the oral cavity. Significance was set as p-value < 0.05.

RESULTS

The mean age of the patients was 37.6 +/- 13.2 years with an age range of 18 to 75 years. Of the total 140 male participants, all participants had a history of tobacco usage in both forms ie; smoking as well as chewing. Maximum participants belonged to the 21–30 years age group (**30.71%**, 43/140), followed by the 31–40 years age group (**25.0%**, 35/140) and the 41–50 years age group (**23.57%**, 33/140).
Among the 70 smokers, **62.85%** (44/70) smoked less than 10 cigarettes per day, while **32.85%** (23/70) smoked 11–20 cigarettes daily. Only **4.28%** (3/70) smoked more than 20 cigarettes per day, indicating that the majority were light to moderate smokers. (Figure 1).

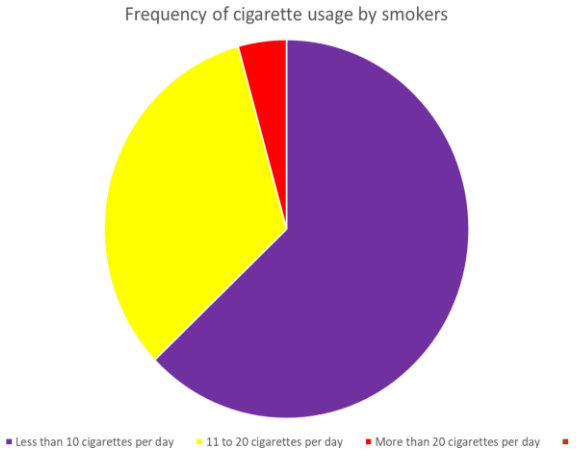


Figure 1. Graphical representation depicting the frequency of smoking tobacco(cigarettes) (No. of times per day)
Of the total 70 chewers, 93% of the participants chewed tobacco around 1-9 times a day and 7% chewed tobacco 10-15 times a day (Figure 2).

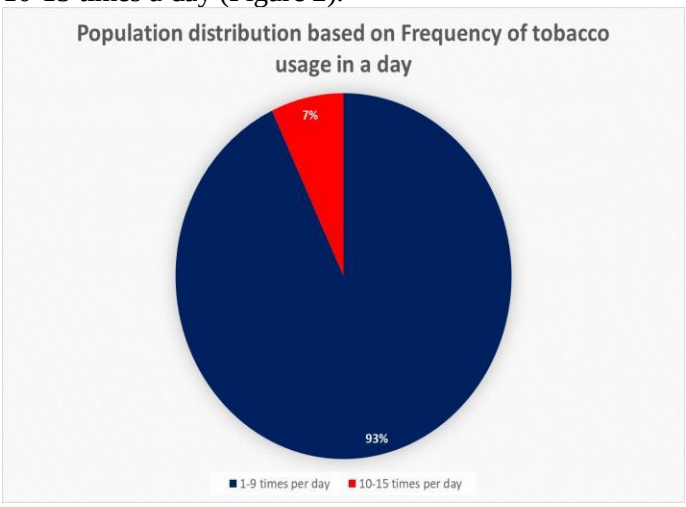


Figure 2. Graphical representation depicting the frequency of chewing tobacco (No. of times per day)

Age-wise distribution of Fagerstrom score among smokers and non-smokers
Smokers: Participants with a nicotine dependency score of 3 were predominantly in the 31–40 years (**10.0%**, 7/70) and 41–50 years (**8.57%**, 6/70) age groups. This highlights that middle-aged smokers (31–50 years) largely fell into the low-dependency category.
Chewers: Among chewers, the highest nicotine dependency score of 5 was observed in the 21–30 years age group (**10.0%**, 7/70), indicating a medium dependency level.

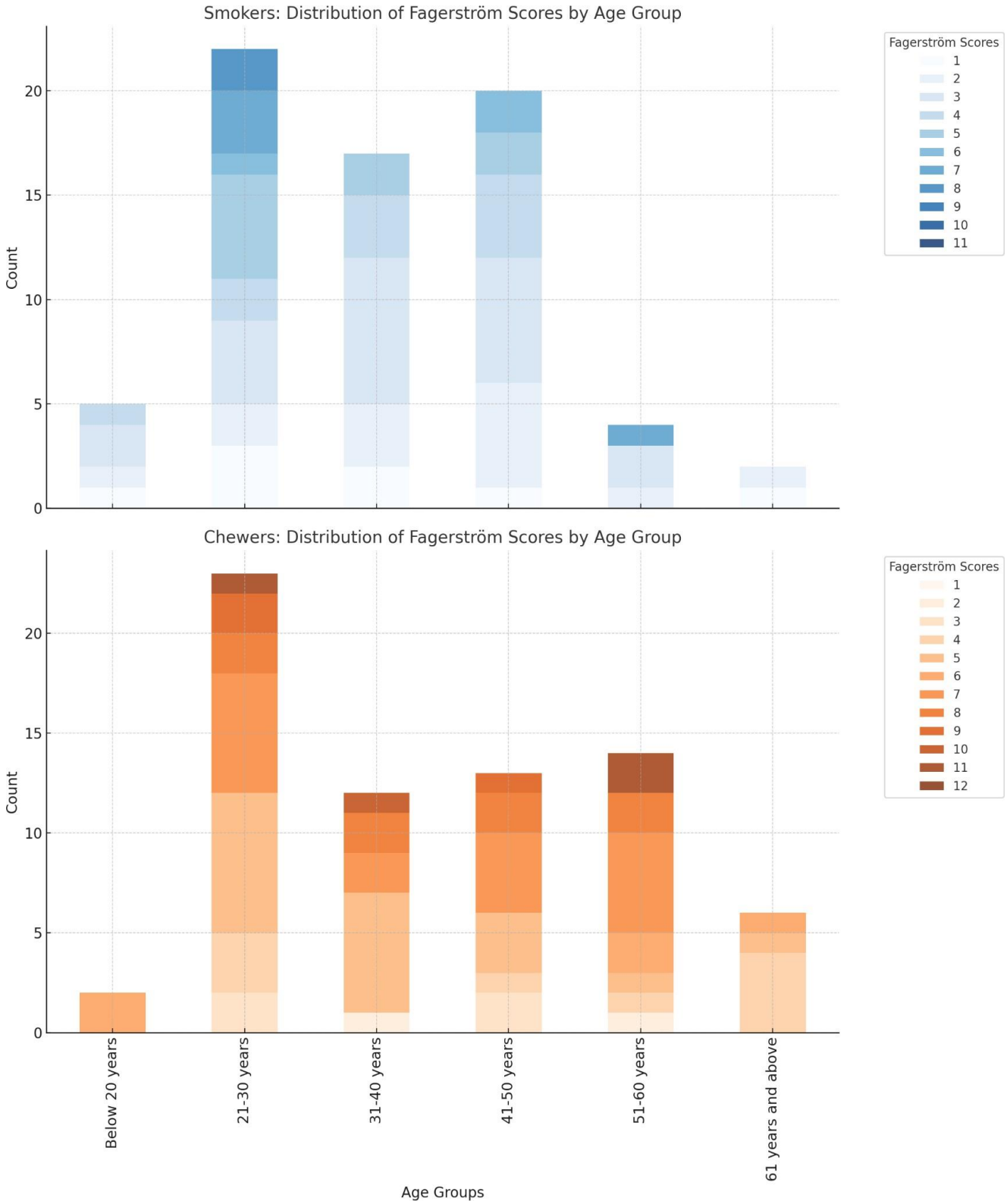


Figure 3 Age-wise distribution of Fagerstrom score among smokers and chewers

Descriptive statistics of Fagerstrom scores among smokers and chewers

The mean age of participants in both categories was 37.66 with a standard deviation of 13.2. The average Fagerstrom score observed was 4.78 +/- 2.304 among both the categories of smokers and chewers.The mean FTND scores among smokers was 3.49 +/- 1.767 and among chewers is 6.07 +/- 2.045 (Table 1&2)

	Mean	Std. Deviation	N
Age	37.664	13.2005	140
Tobacco cessation form (Fagerstrom score)	4.78	2.304	140

1.Smokers/ 2.Chewers		N	Minimum	Maximum	Mean	Std. Deviation
1	**Tobacco cessation form (Fagestorm score among smokers)**	**70**	**1**	**8**	**3.49**	**1.767**
	Valid N (listwise)	**70**				
2	**Tobacco cessation form (Fagestorm score among chewers)**	**70**	**2**	**11**	**6.07**	**2.045**
	Valid N (listwise)	**70**				

Table 1&2: Descriptive statistics

Age Wise distribution of presence and absence of mucosal changes among smokers and chewers

Smokers: Of the 70 smokers, **25.71%** (18/70) presented with oral mucosal lesions, with the majority (**27.78%**, 5/18) found in the 31–40 years age group.

Chewers: Among the 70 chewers, **44.28%** (31/70) exhibited oral mucosal changes. The highest prevalence was in the 21–30 years age group (**32.26%**, 10/31), followed closely by the 51–60 years group (**29.03%**, 9/31). (Figure 4).

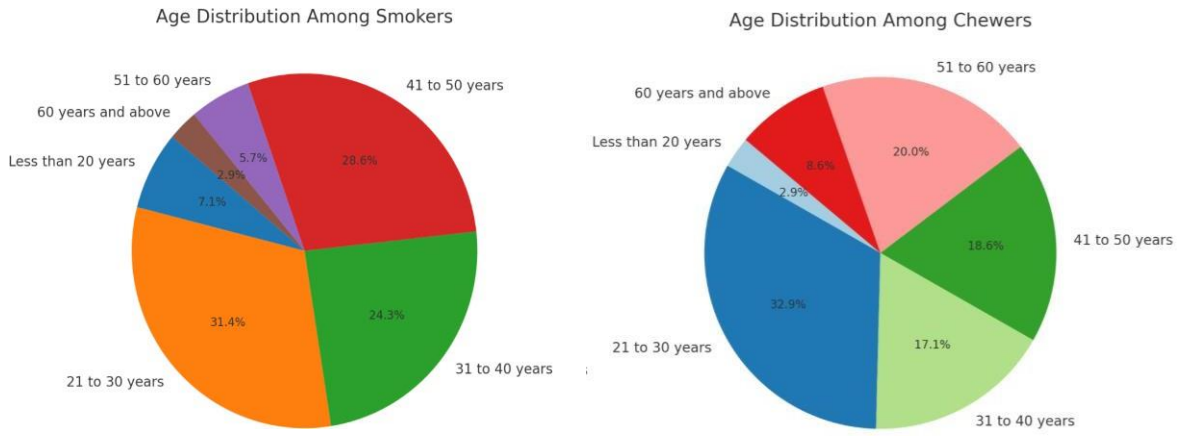


Figure 4. Age Wise distribution of presence and absence of mucosal changes among smokers and chewers

Oral Lesions:

In smokers, the most frequently observed lesions were smoker's palate(figure 5) (83.33%, 15/18) and leukoplakia(figure 6) (27.78%, 5/18). Among chewers, all participants with lesions (100%, 31/31) exhibited tobacco pouch keratosis(figure 7). Additionally, leukoplakia was present in 5.0% (7/140) of participants, observed in both smokers and chewers, while o ral submucous fibrosis (OSMF) was noted in 1.% (2/140) of participants.





Figure 5. Clinical image showing Smoker's palate
Figure 6. Clinical image showing Tobacco pouch keratosis of lower labial mucosa



Figure 7. Clinical images showing Leukoplakia on the buccal mucosa

Pearson's correlation

There is a weak, positive correlation between Age and Mucosal Changes (r = 0.089). This correlation was not statistically significant (p = 0.293). This suggests that there is no reliable linear relationship between age and mucosal changes in this dataset.

There is a very weak, negative correlation between Mucosal Changes and Tobacco Cessation Form (r = -0.279). This correlation was statistically significant at the 0.01 level (p = 0.001). This indicates that individuals with higher mucosal changes tend to have low to intermediate scores on the tobacco cessation form (Fagerstrom Score).

There is a very weak, negative correlation between Age and Tobacco Cessation Form (r = -0.074). This correlation is not statistically (p = 0.386). This suggests that there is no reliable linear relationship between age and tobacco cessation form scores in this dataset (Table 2).

Correlations

Table 2: Correlation statistics

		Age	Mucosal changes	Tobacco cessation form (Fagerstrom score)
Age	Pearson Correlation	1	.089	-.074
	Sig. (2-tailed)		.293	.386
	N	140	140	140
Mucosal changes	Pearson Correlation	.089	1	-.279**
	Sig. (2-tailed)	.293		.001
	N	140	140	140
Tobacco cessation form (Fagerstrom score)	Pearson Correlation	-.074	-.279**	1
	Sig. (2-tailed)	.386	.001	
	N	140	140	140

**. Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

The **Fagerström Test for Nicotine Dependence (FTND)** is a widely used tool to assess nicotine addiction. It evaluates cigarette consumption, the urge to smoke, and overall dependence, scoring responses from 0 to 10. [15, 16]. This tool is particularly useful in determining the intensity of nicotine dependence and guiding cessation strategies [17]. A study among the Narikuravar tribal population in Thoothukudi district found that over 60% of adults used tobacco, with medium nicotine dependency being prevalent. Gum bleeding and tooth erosion were frequently reported among users, emphasizing the need for targeted awareness programs for this vulnerable population [18]. The present study, however, observed that medium dependence was predominantly found among younger chewers (aged 21–30 years), highlighting the specific vulnerability of this age group.

Similarly, Nalini et al.'s systematic review highlighted a lack of research on FTND scores for beedi smokers and smokeless tobacco users in India, with only one study addressing this gap [19]. This finding is consistent with the present study, where both smoking and chewing tobacco habits were examined, emphasizing the importance of filling this research gap.

A rural study in the Kancheepuram district revealed that 48.7% of tobacco users exhibited high nicotine dependence, with the duration of use and age of initiation identified as significant risk factors [20].

The present study showed lower dependency levels among smokers, with most participants in the low-dependence category (FTND score = 3). This contrast may be attributed to differences in population demographics or awareness levels.

In Bhimavaram, reverse smoking, predominantly among older females, was associated with smoker's palate and carcinomatous lesions. Conventional smoking, prevalent among males, was linked to leukoplakia and tobacco-associated melanosis. Chewing tobacco was common among younger males and associated with conditions such as tobacco pouch keratosis and oral submucous fibrosis [21]. The present study corroborates these findings, reporting smoker's palate and leukoplakia among smokers, and tobacco pouch keratosis among all chewers with lesions.

A study in Bagalkot found that 80% of participants had stage I OSMF, and FTND was beneficial in assessing smokeless tobacco dependence. However, no significant correlation was found between FTND scores and OSMF stages due to the small sample size [22]. In the present study, OSMF was observed in only two participants, limiting comparisons. However, the study confirmed the utility of FTND in evaluating nicotine dependence.

A systematic review on the psychometric properties of FTND found uncertainties in its application across cultural groups and in predicting habits among light smokers, e-cigarette users, and smokeless tobacco users. It emphasized the need for improving the psychometric qualities of FTND [23]. The present study aligns with these findings, as the tool proved effective in evaluating dependence among both smokers and chewers but highlighted the need for culturally specific adaptations.

A cross-sectional study in Nepal reported high nicotine dependence among tobacco chewers, correlating with prolonged usage, low socioeconomic status, and failed quit attempts [24]. Similarly, the present study observed a moderate negative correlation between mucosal changes and FTND scores ($r = -0.279$, $p = 0.001$), indicating intermediate dependency levels among those with mucosal changes.

In Goa, a study assessing exhaled carbon monoxide levels found a strong correlation with nicotine dependency and oral mucosal lesions. However, it noted limitations such as self-reported data and environmental factors influencing CO levels [25]. The present study similarly reported a significant association between mucosal changes and tobacco habits but lacked data on CO levels, suggesting an area for future exploration.

A study assessing the effectiveness of the STAR scoring system in oral surgical documentation emphasized the importance of high-quality records for patient follow-up [26]. This underscores the importance of systematic and detailed data collection which paved way for the current research to be carried out.

A prevalence study in Modinagar, India, found a 16.8% occurrence of oral mucosal lesions (OML), with smoker's palate being the most common. It recommended targeted oral health education for high-risk groups [27]. The present study supports this, reporting similar findings of smoker's palate and leukoplakia among tobacco users.

In Dharwad, a higher prevalence of OML (26.8%) was observed among tobacco users compared to non-users (2.8%), with lesions like leukoplakia and OSMF strongly linked to cancer risk [28]. The present study concurs, showing a strong association between tobacco habits and lesions such as smoker's palate, leukoplakia, and tobacco pouch keratosis.

A systematic review reported a significant association between tobacco use and oral premalignant lesions, with an odds ratio of 15.22 for users compared to non-users [29]. This aligns with the present study, which found a higher prevalence of mucosal changes among tobacco users, reinforcing the need for targeted interventions. Heavy Smoking Index (HSI), a shorter two-item version of the FTND, is designed for broader population use. A study conducted in the Galician region of Spain involving 1655 daily smokers compared the validity of the HSI against the FTND. The results demonstrated substantial agreement between the HSI and FTND ($\kappa = 0.7$) and high specificity ($Sp = 96.2\%$), however the sensitivity analysis among females were inconsistent. Thus FTND remains more suitable for populations with lower nicotine dependence, such as women [30].

Limitations of the study include the exclusive focus on male participants, which may limit generalizability. Additionally, as a single-center study, the findings may not apply universally, and the retrospective design restricts control over confounding factors. Additional number of participants among various age groups and an equal number of participants for each category of nicotine dependence can give more data on the correlation aspect. Future research could explore the influence of other factors, such as socioeconomic status, oral hygiene practices, and systemic health conditions, on the development of mucosal changes in tobacco users.

CONCLUSION

From the findings of the current study it can be observed that among smokers, smokers palate is the most common form of lesion noted and is observed even in cases of low dependency. Leukoplakia is also noted among smokers and the associated Fagestorm nicotine dependence score is 6 and 7 which corresponds to High dependence. Among chewers, tobacco pouch keratosis is the most common form of lesion noted, however, the associated score ranges from 3 (Low dependence) to 11

(Very high dependence), also the highest number of patients with lesions belonged to Medium and High dependence categories rather than very high dependence. This could be due to less number of patients recorded under the very high dependency category. One more reason could be because all these scoring is done based on self-reported assessment which can always be manipulated from the participant's end, thereby leading to lower scores in patients with lesions.

In the Fagerstrom scale for nicotine dependence (smokeless), there are questions such as number of hours of chewing per day, length of dipping time per day, how many minutes each dip lasts and number of times used per week, at times few participants who use dips for 10 to 15 times per day have mentioned that they use tobacco less than 2 times per week. These factors can contribute to low and medium scores on occasions were a high or very high score is anticipated. Hence this question regarding the number of times used per week can be modified in terms of question or the choice of answers (choice given in the scale: Less than 2 times, More than 2 times, More than 4 times).

Contributions

NishaJaisree, Pratibha Ramani and Meignana Arumugham have made substantial contributions to the conception and design of the work, the acquisition, analysis, and interpretation of data; and have drafted the work or substantively revised it. NishaJaisree, Pratibha Ramani and Meignana Arumugham have approved the submitted version (and any substantially modified version that involves the author's contribution to the study); NishaJaisree, Pratibha Ramani and Meignana Arumugham have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

DECLARATIONS

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Consent for publication

Informed consent was obtained from every participant for documentation and examination.

Competing interests

The authors declare no competing interests.

Ethical approval

Ethical approval was granted by the Institutional Human Ethical Committee under reference number: IHEC/SDC/DS/FACULTY/23/OPATH/141.

Informed patient consent

All patients' clinical records were obtained with informed consent.

Clinical trial registration details

A clinical trial number is not applicable. It is only an observation of clinical records. This study did not use any clinical intervention or treatment procedures on patients.

CONCLUSION

Patients together with workflow efficiency experienced significant improvement in dental practice through AI-driven virtual assistants. Thorough examination of quantitative alongside qualitative information in the study showed major enhancements affecting domains from patient satisfaction results to clinical results as well as appointment bookings and administrative performance time alongside operational workflow efficiency. The research demonstrates how AI technology will transform dental practice management to give clinicians enhanced power for delivering quality healthcare with optimized operational performance. The identification of patient satisfaction score and staff attitude predictors offers important directions for deploying strategies that yield optimal support for continuous improvement.

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ORIGINAL RESEARCH

INTELECTIN-1 AS A DIAGNOSTIC BIOMARKER FOR NONALCOHOLIC FATTY LIVER DISEASE

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ABSTRACT

In this study, non-alcoholic fatty liver disease (NAFLD) is characterized by the accumulation of fats in the liver cells of individuals who abstain from alcohol consumption. Here, the objective was to assess the levels of intelectin-1, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), insulin, random blood sugar (RBS), total protein, albumin, globulin, triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) in the serum of NAFLD patients. Also, the study investigated the changes in intelectin-1 and other biochemical parameters' ratios in patients and compared them to healthy subjects. It was also determined if alterations in intelectin-1 levels can serve as a diagnostic predictor of NAFLD. 90 volunteers were involved in this study, among them 60 who were patients diagnosed with NAFLD at Baquba Teaching Hospital, Diyala Province, Iraq, between January 10, 2022, and December 30, 2022. The other 30 were healthy individuals who were recruited as a control group. It was found that levels of intelectin-1 in serum were significantly higher in NAFLD patients compared to healthy individuals ($p < 0.05$). Significant elevations ($p < 0.05$) were observed in the mean values of ALT and AST in patients compared to controls. Levels of insulin, ALP, total protein, Albumin, RBS, and Globulin showed non-significant elevations in patients compared to controls ($p > 0.05$). Serum levels of TC, TG, LDL, and VLDL demonstrated substantial and significant elevations ($p < 0.05$) in patients compared to controls, while HDL levels were reduced, albeit non-significantly. We found that intelectin-1 levels exhibited the highest sensitivity and area under the curve (AUC) in the receiver operating characteristic curve compared to other biochemical factors. Thus, an increase in intelectin-1 levels that are accompanied by the elevation of TG, TC, LDL, VLDL, and liver function parameters can serve as a predictor in diagnosing NAFLD patients.

Keywords**:** NAFLD; intelectin-1; insulin; lipids; RBS; total protein

INTRODUCTION

Globally, Nonalcoholic fatty liver disease (NAFLD) is one of the largest public liver diseases[1]. NAFLD is characterized by the accumulation of triglycerides in hepatic cells in individuals who abstain from alcohol consumption. This condition initially exhibits no indications of liver cell inflammation or damage [2]. If not identified or addressed in its initial phases, NAFLD can progress to liver cirrhosis, a grave condition that may lead to mortality. The disease is frequently associated with other health conditions, including type 2 diabetes, hypertension, obesity, metabolic syndrome, and cardiovascular complications [3].

Hepatic insulin resistance in patients with NAFLD impairs the ability of insulin to suppress glucose production, causing mild hyperglycemia. This stimulation could increase insulin secretion and result in hyperinsulinemia. The metabolic disruptions are intricately connected to the buildup of fat in the liver,

even when the body mass index (BMI) is not elevated [4]. Here, pro-inflammatory cytokines play a pivotal role in the progression of fatty liver disease [5].

Adipokines are a group of regulatory proteins derived from adipose tissue that play diverse roles across the central, local, and peripheral systems [6]. These molecules are notably important in the context of fatty liver disease, as their concentrations vary between individuals with the condition and those without it [7]. Intelectin-1, also referred to as Omentin-1, is a type of adipokine. It is a polypeptide hormone that is produced and released primarily by visceral fat tissue. It can be found surrounding the internal organs, such as the liver, intestines, and kidneys [8]. Alteration of intelectin-1 expression is associated with several chronic inflammatory illnesses [9]. Intelectin-1 increases insulin sensitivity and glucose uptake [10] and alters the metabolism of fat [11]. Hence, the levels of insulin and glucose affect the intelectin-1 level[12]. Recent research has identified associations between the high and low concentrations of intelectin-1 and a broad spectrum of chronic inflammatory disorders [13].

In our work, we compared the concentrations of intelectin-1 and other biomarkers measured in patients with NAFLD to those of the healthy control. In addition to this, we aimed to find whether or not changes in intelectin-1 levels could be used as a marker for identifying patients with NAFLD.

MATERIALS AND METHODS

2-1 Collection of samples

Blood samples of a total number of 90 were collected between October 1st, 2022, and December 30th, 2022, at Baquba Teaching Hospital in Diyala Governorate/Iraq. Among these samples, 60 were obtained from NAFLD patients diagnosed via ultrasound examination conducted by a specialist physician. These patients were 31 males and 29 females, aged from 30 to 70 years. The other samples (30) were taken from "apparently" healthy individuals who participated as the control group and aged between 30 and 70 years. This group was divided equally based on gender classification, 15 males and 15 females. Each participant provided 5 ml of venous blood utilizing polymeric sterile medical syringes. The drawn blood samples were then set in plain tubes and allowed to clot for 10-30 minutes. The serum was separated by centrifugation for 5 minutes at 3000 rpm. Each serum sample was divided into three parts: the first part was used for the estimation of cholesterol, triglycerides (TG), aspartate aminotransferase (AST), high-density lipoprotein (HDL), alkaline phosphatase (ALP), alanine aminotransferase (ALT), albumin (ALB), random blood sugar (RBS), globulin, and total protein. The second sample was allocated for insulin analysis, while the third was preserved at −20 °C in a deep freezer until required for the ELISA-based quantification of Intelectin-1. Anthropometric measurements were obtained, encompassing age, height, weight, and waist circumference. The body mass index (BMI) was calculated using the formula: weight (kg) divided by height (m) squared.

2-2 Analysis of clinical parameters

To determine the concentration of Intelectin-1 in serum samples, an ELISA kit manufactured by Human Company (Germany) was utilized. Serum insulin levels were assessed using the HS-INSULIN-CHECK-1 rapid quantitative assay. Commercial kits from Human Company (Germany) were also used to measure AST, ALT, ALP, albumin, globulin, and total protein. Additionally, serum levels of random blood sugar (RBS), triglycerides (TG), total cholesterol (TC), and high-density lipoprotein (HDL) were analyzed with the aid of a German-made COBS 411 automated chemical analyzer. The low-density lipoprotein (LDL)-Cholesterol and very low-density lipoprotein (VLDL)-Cholesterol were derived using the Friedewald equation, as follows: LDL-Cholesterol = [Total Cholesterol] - [HDL-Cholesterol] - ([Triglycerides]/5), VLDL-Cholesterol = [Triglycerides]/5.

2-3 Statistical analysis

The statistical analysis was performed using SPSS software version 25, The results were presented as mean values accompanied by standard deviations. To assess the significance of differences between variable factors, an independent chi-square test and t-test were employed. Here, a significance level of $p < 0.05$ was considered statistically significant. Pearson correlations were established using the t-test to determine the significance of correlations between two quantitative variables.

3.RESULTS

The study results revealed significant differences ($p < 0.05$) in age, body mass index (BMI), and waist circumference between the two studied groups. The mean values for age, BMI, and waist circumference were higher in patients (46.78 ± 12.30 years, 31.79 ± 5.72 kg/m^2, and 108.33 ± 16.48 cm, respectively) compared to healthy individuals (40.07 ± 10.05 years, 26.22 ± 5.57 kg/m^2, and 92.21 ± 11.41 cm, respectively), as presented in Table 1.

Table 1. Comparison of Age, BMI, and Waist Circumference between Nonalcoholic Fatty Liver Disease Patients and Healthy Individuals

Groups		N	Mean ± Std. Deviation	P value
Age (years)	Patients	60	46.78 ± 12.30	p<0.05*
	Healthy	30	40.07 ± 10.05	
BMI (Kg/m^2)	Patients	60	31.79 ± 5.72	P<0.05*
	Healthy	30	26.22 ± 5.57	
Waist (cm)	Patients	60	108.33 ± 16.48	p<0.05*
	Healthy	30	92.21 ± 11.41	

*= significant, **= high significant, ***= very high significant

3-2 Anthropometries of Fatty Liver patients and the healthy group

As previously stated, the age range of the nonalcoholic fatty liver disease patients was 30-70 with an average of 46.78 ± 12.30. The results indicate that nonalcoholic fatty liver disease incidence rises along with age for both genders, but this enhancement is not statistically significant (P > 0.05). Nevertheless, males comprise the majority of affected NAFLD patients, accounting for 53.3% compared to 46.7% of females. Regarding gender and age groups, the current findings suggest no remarkable differences (P > 0.05) between age and gender groups within the studied groups. However, based on BMI levels, high differences (P ≤ 0.05) were observed between the patients and healthy groups. The majority of patients (90.0%) fell within the obese weight BMI category, whereas the majority of healthy individuals (70.0%) fell within the normal weight BMI category. Table 2 illustrates these findings.

Table 2. Comparative Anthropometric Merits of Participants Using the Chi-Square Test

			Groups		Total	P value
			Patients	Healthy		
Gender	Male	N	32	15	47	p>0.05
		%	53.3%	50.0%	52.2%	
	Female	N	28	15	43	
		%	46.7%	50.0%	47.8%	
Age group (years)	30-39	N	15	18	39	p>0.05
		%	25.0%	60.0%	43.3%	
	40-49	N	13	6	19	
		%	21.7%	20.0%	21.1%	
	50-59	N	21	5	20	
		%	35.0%	16.7%	22.2%	
	60-70	N	11	1	12	
		%	18.3%	3.3%	13.3%	
BMI	Normal	N	6	21	15	P<0.05*
		%	10.0%	70.0%	16.7%	
	Obese	N	54	9	75	
		%	90.0%	30.0%	83.3%	

*= significant, **= high significant, ***= very high significant

3-3 Comparison of liver function factors in the investigated groups

The results indicate significant differences (p < 0.05) between the ALT and AST biomarkers among the studied groups. The mean values for ALT and AST were higher in patients (28.51 ± 12.78 and 32.23 ± 10.36, respectively) compared to the healthy group. However, no significant differences (p > 0.05) were observed for ALP and albumin levels between the studied groups. Our findings in this regard are summarized in Table 3.

Table 3. Comparative Liver Function Factors Between Studied Groups Using T-Test

Groups		N	Mean± Std. Deviation	P value
ALT (U/L)	Patients	60	28.51±12.78	p<0.01**
	Healthy	30	18.05±5.72	
AST (U/L)	Patients	60	32.23±10.36	p<0.01**
	Healthy	30	23.05±6.80	
ALP (U/L)	Patients	60	186.68±53.82	p>0.05
	Healthy	30	182.16±47.20	
Albumin (g/dl)	Patients	60	3.31±0.65	p>0.05
	Healthy	30	3.67±0.35	

*= significant, **= high significant, ***= very high significant

3-4 Comparison of RBS and lipid profile between the investigated groups

The study results revealed significant differences ($p < 0.05$) in cholesterol, TG, VLDL, and LDL levels when comparing between the studied groups. The mean values of cholesterol, TG, VLDL, and LDL were significantly higher in patients (237.70 ± 50.93, 337.62 ± 152.09, 67.52 ± 30.42, and 132.31 ± 53.93, respectively) than in healthy controls (194.10 ± 29.80, 197.00 ± 42.07, 49.83 ± 25.41, and 104.44 ± 33.26, respectively). HDL and RBS levels were decreased in patients compared to healthy controls, but the decreases were without significant differences ($p > 0.05$) between the studied groups as depicted in Table 4.

Table 4. Comparative RBS and Lipid Profile Parameters Between Studied Groups Using T-Test

Groups		N	Mean± Std. Deviation	P value
RBS(mg/dl)	Patients	60	150.86 ± 78.54	p>0.05
	Healthy	30	155.27 ± 72.13	
Cholesterol (mg/dl)	Patients	60	237.70±50.93	p<0.001***
	Healthy	30	194.10±29.80	
TG (mg/dl)	Patients	60	337.62±152.09	p<0.001***
	Healthy	30	197.00±42.07	
VLDL (mg/dl)	Patients	60	67.52±30.42	p<0.01**
	Healthy	30	49.83±25.41	
HDL (mg/dl)	Patients	60	36.98±5.03	p>0.05
	Healthy	30	40.77±5.92	
LDL (mg/dl)	Patients	60	132.31±53.93	p<0.001***
	Healthy	30	104.44±33.26	

*= significant, **= high significant, ***= very high significant

3-5 Comparison of proteins, globulin, insulin, and Intelectin-1 in studied groups

The results did not reveal significant differences ($p > 0.05$) in the values of RBS, total protein, globulin, and insulin between the two studied groups. Although these parameters showed increases in patients compared to controls, these increases were not statistically significant ($p > 0.05$). However, the mean value of intelectin-1 was significantly higher in patients (1.00 ± 0.29) compared to healthy controls, with a p-value less than 0.05, indicating a statistically significant difference. These outcomes are summarized in Table 5 below.

Table 5. A comparison of the Protein, Globulin, Insulin, and Intelectin-1 Levels Between Studied Groups Using the T-Test

Groups		N	Mean± Std. Deviation	P value
Protein (g/dl)	Patients	60	7.93±0.17	p>0.05
	Healthy	30	7.86±0.22	
Globulin (g/dl)	Patients	60	4.62±0.69	p>0.05
	Healthy	30	4.19±0.44	
Insulin (IU/ml)	Patients	60	18.06±5.95	p>0.05
	Healthy	30	16.59±4.39	
Intelectin-1 (ng/mL)	Patients	60	1.10±0.30	p<0.01**
	Healthy	30	0.41±0.25	

*= significant, **= high significant, ***= very high significant

3-6. Receiver Operating Characteristic (ROC) Curve, Sensitivity, and Specificity of Variables

The plotting of the receiver operating characteristic (ROC) curve provides a measure of the investigative power of serological tests, expressed as the AUC. The AUC simplifies the comparison of various tests into a single number. Hence, Table 6 illustrates that intelectin-1 exhibits the highest AUC and sensitivity of 0.921 and 91%, respectively. In contrast, ALT, AST, cholesterol, TG, VLDL, and globulin parameters displayed sensitivities of 73%, 75%, 74%, 83%, 70%, and 73%, respectively, in screening patients. These sensitivities were significantly higher ($p < 0.05$) compared to ALP, RBS, and HDL (28%, 44%, and 36%, respectively).

Focusing on the specificity, ALB and HDL demonstrated the highest values (76% and 60%, respectively) compared to globulin, insulin, AST, cholesterol, and VLDL, where they were 30%, 43%, 36%, 43%, and 43%, respectively, with significant differences observed ($p < 0.05$).

Table 6. The ROC Curve with Sensitivity and Specificity of All Variables

Test Result Variable(s)	Area	Std. Error	P value	Sensitivity %	Specificity %
ALT	0.728	0.054	p<0.001***	73	46
AST	0.749	0.055	p<0.001***	75	36
ALP	0.523	0.063	p>0.05	53	45
ALB	0.288	0.056	p<0.001***	28	76
RBS	0.430	0.061	p>0.05	44	56
Cholesterol	0.762	0.050	p<0.001***	74	43
TG	0.829	0.042	p<0.001***	83	50
VLDL	0.703	0.060	p<0.01**	70	43
HDL	0.381	0.066	p>0.05	36	60
LDL	0.654	0.057	P<0.05*	66	46
Protein	0.613	0.067	p>0.05	58	53
Globulin	0.724	0.055	p<0.001***	73	30
Insulin	0.559	0.066	p>0.05	56	43
Intelectin-1	0.921	0.032	p<0.001***	91	26

*= significant, **= high significant, ***= very high significant

DISCUSSION

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.

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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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

ASSESSING S100A7 AS A DIAGNOSTIC BIOMARKER IN ORAL CANCER PATIENTS

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) is a worldwide issue, and its outlook is poor if detected late. At present, most diagnosis depends on examining tissues, which can be unreliable because different experts may see things differently. Scientists suggest that S100A7, a calcium-binding protein, may be used to identify different malignancies. The meaning of S100A7 expression in distinguishing healthy oral glands, OPMDs, and OSCC was investigated in this study.

Material and Methods: Retrospective analyses were made on tissue samples taken from 245 individuals: 89 with OSCC, 78 with OPMD, and 78 people who had normal oral mucosa. Immunohistochemical staining for S100A7 was carried out according to standardized steps. We looked not only at how much the cells stained, but also how many cells were stained for each marker. Data was analyzed with chi-square tests, ROC curves, and multivariate logistic regression.

Results: The S100A7 gene was actively expressed in 84.3% of OSCC samples, 61.5% of OPMDs, and 12.8% of normal skin tissues ($p<0.001$). In 76.4% of OSCC cases, there was strong S100A7 staining, but only 23.1% of OPMD cases showed this pattern. The ROC curve covered 84.2% for the purpose of telling OSCC apart from OPMDs. Nuclear and cytoplasmic S100A7 levels were strongly linked to tumor grade and lymph node metastasis ($p<0.05$).

Conclusion: This biomarker has valuable use for finding oral cancer and grouping patients by their risk levels. Because CEA is expressed at higher levels in cancerous tissues and is linked to worse tumor signs, it is useful in both reviewing and planning treatments for patients with oral cancer.

Keywords*:* S100A7, oral squamous cell carcinoma, biomarker, immunohistochemistry, oral potentially malignant disorders, diagnostic marker

1. INTRODUCTION

About 90% of head and neck malignancies, which is the seventh most common cancer, are called oral squamous cell carcinoma (OSCC)[1,2]. The greatest number of cases occurs in South and Southeast Asia, largely because many people there use carcinogenic substances such as areca nut and tobacco products. Although there are more options for treating OSCC, five-year survival is often poor for those diagnosed with the cancer at an advanced stage[3,4].

Histopathological examination of biopsy tissue is currently used to diagnose OSCC, and this process involves looking at cells and their surroundings and making a subjective interpretation of what is seen[5,6]. Such an approach is well-known, but still comes with problems: inter-observer disagreements and uncertain prediction of OPMDs' malignancy chance[7,8]. The old system and the new suggested system have both shown significant differences in how consistently pathologists use them for diagnoses.

There is now a major focus on finding dependable biomarkers to enhance how oral cancer is diagnosed and what the outcome will be[9–11]. Many scientists have been paying special interest to S100 proteins, as they are involved in cell growth, differentiation, death, and inflammation[12,13]. Among the S100 family are over 20 proteins, several of which are involved in both the creation and advancement of cancer[14].

S100A7, another name for psoriasin, is a small protein that binds calcium and largely appears in cells called keratinocytes[2,7]. Under usual conditions, S100A7 plays a key part in both skin differentiation and the body's immune system[7]. Still, abnormal amounts of S100A7 are known in many cancers, including those of the breast, cervix, and skin[2,15]. Oral cancers have shown S100A7 to promote cell division, movement, invasion, and metastasis using different signaling networks[3].

Research done recently shows that the expression of S100A7 is much higher in oral squamous cell carcinomas than in normal oral tissues[1,5]. Moreover, raised levels of S100A7 tend to correlate with the characteristics of tumors, the presence of lymph node metastasis, and a patient's survival chances[1,3]. They show that S100A7 could be a useful diagnostic and prognostic biomarker for patients with oral cancer.

S100A7 helps cause oral cancer by increasing RAGE signaling, promoting EMT, and boosting inflammation[15]. It acts both inside cells and is secreted by them, serving to shape cancer cell behavior and also to affect the environment around the tumor[13].

Though the early outcome shows hope, more studies and comparisons with current tests should be carried out in people with different types of oral cancer. Another important research aim is to establish standard ways of assessing and deciding on appropriate cut-off levels[16].

2. MATERIAL AND METHODS

Study Layout and Choosing Appropriate Patients

The institutional ethics committee approved before the authors conducted this retrospective cross-sectional study at a tertiary care medical center. Patients who had an oral tissue biopsy during the period from January 2020 to December 2023 were part of the study population. Researchers included patients if they were over 18 years old, had histologically confirmed OSCC, OPMD, or normal oral mucosa, had some FFPE tissue sections, and had complete data available. Criteria used to exclude patients included: (1) having had oral cancer treatment before, (2) existing cancer elsewhere, (3) low quality of tissue samples, and (4) missing important clinical information.

Sample Collection and Processing

Our research included tissue samples collected by incisional or excisional biopsies done by experienced oral and maxillofacial surgeons. Each specimen was preserved in 10% neutral buffered formalin for at least a day and up to two days and then processed for paraffin embedding using general histopathology methods. Thin slices of 4-µm thickness were made on the rotary microtome and added to glass slides for the following staining methods[17–20].

Histopathological Evaluation

A standard H&E staining process was used on some tissue sections to help make the histopathological diagnosis. No clinically linked information was shared with the oral pathologists, and each specimen was looked at by them individually. The types of lesions reviewed were (1) healthy oral mucosa, (2) inflammatory problems, (3) potentially malignant disorders in the mouth (such as leukoplakia, erythroplakia, lichen planus, and epithelial dysplasia), and (4) oral squamous cell carcinoma. WHO classification was used to grade dysplastic lesions as mild, moderate, or severe dysplasia[1,5].

Steps for Immunohistochemical Staining

Using protocols described in previous reports[1,7], testing for the presence of S100A7 protein was carried out by immunohistochemical analysis. The specimens were first given a xylene bath and then cleaned with graded alcohol solutions. Eight hundred watts of microwave energy were used for 10 minutes, along with 480 watts for an additional 5 minutes, to retrieve the antigens with citrate buffer at pH 6.0. To block peroxidase activity, we left the samples in 0.3% hydrogen peroxide in methanol for 30 minutes[1,20].

For 16 hours, a humidified chamber was used to house the primary mouse monoclonal anti-S100A7 antibody, diluted 1:200 and applied overnight at 4°C.

Memory T cells were left in the primary antibody for 30 minutes and then washed. The next step was to incubate these cells with biotinylated secondary antibody for 30 minutes at room temperature. Staining was performed using diaminobenzidine (DAB) to visualize, and sections were stained with hematoxylin afterward[7,16].

Checking the amount of S100A7 production

An independent semi-quantitative scoring method for S100A7 immunostaining was applied by two pathologists. Part of the assessment was to look at: (1) number of tumor cells (0: <5%, 1: 5-25%, 2: 26-50%, 3: 51-75%, 4: >75%) and (2) how intense the staining was (0: negative, 1: weak, 2: moderate, 3: strong). The composite score was found by multiplying the percentage score by the intensity score and resulting in a score between 0 and 12. A S100A7 score of ≥4 was labeled as positive for expression.

Each type of staining was registered by itself, because studies have indicated that where the protein is within the cell can have different effects. Data from regions affected by inflammation and cell death were left out so that the analysis of epithelial cell staining could be precise.

Statistical Analysis

SPSS version 26.0 was used for the statistical analysis. Chi-square tests or Fisher's exact test were used to compare categories of variables. Variables that changed along a continuum were analyzed using Student's t-test, where data were normally distributed, and the Mann-Whitney U test, where data were not normally distributed. A receiver operating characteristic curve was used to test how well S100A7 helps distinguish between various tissue categories. Multivariate logistic regression was used to discover the factors that predict malignant transformation in adenomas. In our analysis, results were considered meaningful when the P-value was less than 0.05, and all tests were run in both directions.

3. RESULTS

Patient Demographics and Clinical Characteristics

The study included 245 patients with a mean age of 54.2 ± 12.8 years (range: 22-81 years). The study population comprised 89 OSCC patients, 78 OPMD patients, and 78 control subjects with normal or inflammatory oral lesions. Male patients predominated in the study population (n=156, 63.7%) with a male-to-female ratio of 1.75:1. The demographic and clinical characteristics of the study population are summarized in **Table 1.**

Table 1. Demographic and Clinical Characteristics of the Study Population

Parameter	OSCC (n=89)	OPMD (n=78)	Controls (n=78)	p-value
Age (years)	56.8 ± 11.4	52.1 ± 13.2	53.7 ± 12.9	0.023
Gender				
Male	62 (69.7%)	48 (61.5%)	46 (59.0%)	0.284
Female	27 (30.3%)	30 (38.5%)	32 (41.0%)	
Tobacco Use	76 (85.4%)	54 (69.2%)	28 (35.9%)	<0.001
Alcohol Use	58 (65.2%)	35 (44.9%)	22 (28.2%)	<0.001
Site				
Buccal mucosa	44 (49.4%)	32 (41.0%)	29 (37.2%)	0.156
Tongue	23 (25.8%)	19 (24.4%)	18 (23.1%)	
Gingiva	14 (15.7%)	16 (20.5%)	20 (25.6%)	
Floor of the mouth	8 (9.0%)	11 (14.1%)	11 (14.1%)	

Among OSCC patients, the majority presented with moderately differentiated tumors (n=52, 58.4%), followed by well-differentiated (n=25, 28.1%) and poorly differentiated carcinomas (n=12, 13.5%). Regarding tumor staging, 34 patients (38.2%) presented with early-stage disease (T1-T2), while 55 patients (61.8%) had advanced-stage tumors (T3-T4). Lymph node metastasis was detected in 47 patients (52.8%) at the time of diagnosis.

S100A7 Expression Patterns

Immunohistochemical analysis revealed distinct S100A7 expression patterns across different tissue categories. S100A7 staining was observed in both nuclear and cytoplasmic compartments of epithelial cells, with varying intensities and distribution patterns. The results of S100A7 expression analysis are presented in **Table 2.**

Table 2. S100A7 Expression Patterns in Different Tissue Categories

Tissue Category	Negative (%)	Weak (%)	Moderate (%)	Strong (%)	Total Positive (%)	p-value
Normal/Inflammatory	68 (87.2%)	7 (9.0%)	3 (3.8%)	0 (0%)	10 (12.8%)	<0.001
OPMD	30 (38.5%)	28 (35.9%)	16 (20.5%)	4 (5.1%)	48 (61.5%)	
OSCC	14 (15.7%)	18 (20.2%)	36 (40.4%)	21 (23.6%)	75 (84.3%)	

S100A7 expression was significantly higher in OSCC specimens compared to both OPMD and control groups ($p<0.001$). Strong intensity staining was predominantly observed in OSCC cases (23.6%) and was rare in OPMD cases (5.1%) and absent in control specimens. The subcellular localization patterns also differed significantly between groups, with nuclear staining being more prevalent in malignant tissues.

Correlation with Clinicopathological Parameters

S100A7 expression levels showed significant correlations with various clinicopathological parameters in OSCC patients. The relationships between S100A7 expression and tumor characteristics are detailed in **Table 3.**

Table 3. Correlation of S100A7 Expression with Clinicopathological Parameters in OSCC

Parameter	S100A7 Low (n=35)	S100A7 High (n=54)	p-value
Tumor Grade			
Well differentiated	18 (51.4%)	7 (13.0%)	<0.001
Moderately differentiated	15 (42.9%)	37 (68.5%)	
Poorly differentiated	2 (5.7%)	10 (18.5%)	
T Stage			
T1-T2	20 (57.1%)	14 (25.9%)	0.003
T3-T4	15 (42.9%)	40 (74.1%)	
Lymph Node Status			
Negative	24 (68.6%)	18 (33.3%)	0.001
Positive	11 (31.4%)	36 (66.7%)	
Tumor Size			
≤2 cm	19 (54.3%)	12 (22.2%)	0.002
>2 cm	16 (45.7%)	42 (77.8%)	

High S100A7 expression was significantly associated with advanced tumor grade ($p<0.001$), larger tumor size ($p=0.002$), advanced T stage ($p=0.003$), and presence of lymph node metastasis ($p=0.001$). These findings indicate that S100A7 expression correlates with aggressive tumor behavior and poor prognostic indicators.

Diagnostic Performance Analysis

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic accuracy of S100A7 expression in distinguishing between different tissue categories. The area under the curve (AUC) values and associated statistics are presented in **Table 4.**

Table 4. Diagnostic Performance of S100A7 Expression

Comparison	AUC	95% CI	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
OSCC vs Controls	0.923	0.881-0.965	84.3	87.2	86.2	85.4
OSCC vs OPMD	0.842	0.781-0.903	84.3	61.5	71.4	76.8
OPMD vs Controls	0.789	0.723-0.855	61.5	87.2	82.8	69.4
High-grade OPMD vs Low-grade OPMD	0.736	0.621-0.851	78.9	68.2	65.2	81.1

The highest diagnostic accuracy was observed when comparing OSCC with normal controls (AUC = 0.923), followed by OSCC versus OPMD comparison (AUC = 0.842). These results demonstrate that S100A7 expression has excellent discriminatory ability for detecting malignant transformation and good performance for distinguishing between different stages of oral carcinogenesis.

Multivariate Analysis

Multivariate logistic regression analysis was performed to identify independent predictors of malignant transformation. The analysis included age, gender, tobacco use, alcohol consumption, and S100A7 expression level as variables. S100A7 high expression emerged as an independent predictor of malignancy (OR = 3.42, 95% CI: 1.87-6.25, $p<0.001$), along with tobacco use (OR = 2.18, 95% CI: 1.12-4.24, $p=0.022$) and advanced age (OR = 1.04, 95% CI: 1.01-1.07, p=0.0

4.DISCUSSION

This study shows that S100A7 has value for diagnosing oral squamous cell carcinoma and classifying its risk. We discovered that S100A7 is upregulated in malignant oral tissues, and its strong ability to discriminate these tissues means it could be used in oral cancer diagnosis. S100A7 is seen to be expressed in higher amounts as the disorder becomes more invasive, matching previous research on what the protein does in developing oral carcinoma[1,5]. The reason for these results may indicate that S100A7 is useful for both checking and predicting cancer development in premalignant lesions. Out of 102 OSCC cases, S100A7 expression was positive in 84.3%, which is far higher than the 12.8% of controls, meaning this biomarker can be counted on to detect cancer.

Upregulation of S100A7 in oral cancer happens through various pathways that encourage both the growth and spread of the tumor[2,7]. Both inside cells and in the space around our bodies, S100A7 regulates cell growth, movement, and invasiveness by activating RAGE signaling and epithelial-mesenchymal transition. In addition, the protein can change how inflammation works and recruit macrophages linked to tumors, leading to more oral cancer problems[14,15].

Analysis of the correlation found that increased S100A7 expression tends to be present in poorly differentiated, locally advanced, and metastatic tumors. The same relationship has been seen in other types of cancer, where too much S100A7 is related to worse patient outcomes[3,12]. It is very important to mention the strong link to lymph nodes, as cancer spreading to nodes is a key factor in determining patient outcomes. Therefore, measuring S100A7 could influence both the choice of treatment and how patients are counselled about their condition.

The results from figuring out which S100A7 best distinguished OSCC from normal tissues and OPMDs were impressive, with the area under the ROC curve at 0.923 for the former and 0.842 for the latter. The results of these performance characteristics match or exceed those reported in other biomarkers for oral cancer[9,10]. Since S100A7 immunohistochemistry reaches a sensitivity of 84.3% and specificity of 87.2%, it is likely to be helpful in difficult situations where pathologists might find it hard to confirm OSCC on their own. This ability of S100A7 to separate the high-grade from low-grade OPMDs (AUC = 0.736) has important medical use, since current histopathology methods are often not accurate or consistent for predicting malignancy[4,19,21]. Spotting OPMDs that tend to transform into cancer allows doctors to put patients under closer surveillance and deal with cases earlier, which may result in early cancer detection and better outcomes for the patients.

According to our multivariate results, having high S100A7 levels independently raises your chances of having malignant disease by more than three times compared to those with low S100A7 levels. Therefore, along with other known risk factors, including tobacco use and getting older, the benefits of S100A7 measurement help its inclusion in overall oral cancer risk scoring systems[12,16,22].

Using S100A7 as a diagnostic marker in the clinic is realistically possible since suitable staining techniques and scoring systems are in place[1,16]. Because this method can be applied at most pathology labs using standard equipment, it is suitable for many clinical applications. Even so, having set cut-off values and using quality control methods helps guarantee equal and repeatable outcomes at every facility.

A number of factors in this study should be considered. Because this study recruited from only one center, its results may not apply to all patients who need similar care. The values that separate high and low levels of S100A7 expression in current research should be checked using more data to determine suitable thresholds in clinical practice. Due to the limited size of some subgroup samples, these analyses may not have found subtle but important differences between groups.

More studies are needed to test S100A7 as an effective measure in a large number of representative samples gathered from many centers worldwide. Looking at how S100A7 changes during treatment and after can help understand its use for both monitoring how well therapy works and detecting if the disease comes back[14]. By combining digital pathology and artificial intelligence, new quantitative methods might improve both the consistency and fairness in calculating S100A7 biomarkers.

Assuming S100A7 screening is matched with freshly developed biomarkers and molecular testing, it has the potential to contribute to the accuracy of diagnostic panels. Examining the use of S100A7 with various biomarkers, imaging results, and tissue tests can contribute to designing improved methods for oral cancer detection and the division of patients into risks[3,16].

5. CONCLUSION

In essence, our study proves that S100A7 can be beneficial in detecting oral squamous cell carcinoma and should therefore be clinically useful. Because it is highly expressed in malignant tumors, because its performance in diagnosing oral cancer is strong, and because it goes along with aggressive tumors, its incorporation into oral cancer management might be useful. Being able to tell apart different oral potentially malignant disorders helps doctors plan treatment and assess the risk involved. There is a need for more research on protocols and cut-off values, but S100A7 seems to be a good choice for discovering oral cancer and supports better and more accurate oral cancer care.

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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ORIGINAL RESEARCH

ODONTOGENIC CYSTS AND TUMOURS: CLINICOPATHOLOGICAL DATA OF 13 YEARS FROM TERTIARY ORAL HEALTH CARE CENTER OF SOUTH INDIA

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ABSTRACT

Background: Odontogenic cysts and tumors arise from remnants of the tooth-forming apparatus and exhibit diverse biological behaviors. Accurate diagnosis is essential for effective management, especially with updates in the WHO 2024 classification. To retrospectively analyze the demographic, clinical, radiological, and histopathological features of odontogenic cysts and tumors diagnosed over 13 years at a tertiary oral health center in South India.

Material and Methods: This study reviewed 433 histopathologically confirmed cases from 6,739 oral biopsies recorded between 2010 and 2023. Data were analyzed for age, gender, lesion site, radiographic appearance, and pathological type. Classification was done according to the WHO 2024 guidelines.

Results: A total of 433 cases (6.42%) were identified, with a male predominance (1.6:1) and peak incidence in the 20–29 years age group. The mandible was more frequently affected, particularly on the left side. Odontogenic keratocyst was the most common cyst (121 cases), while ameloblastoma was the most common tumor (97 cases). Most lesions presented as unilocular radiolucencies.

Conclusion: Odontogenic lesions in this population showed trends consistent with global data. The study underscores the value of histopathology and the relevance of the updated WHO classification in guiding diagnosis and management of these diverse lesions.

Keywords: Odontogenic cysts, Odontogenic tumours, Ameloblastoma, Odontogenic keratocyst cyst, Radicular cyst

INTRODUCTION

Odontogenic cysts and tumors are lesions that originate from the epithelial and/or mesenchymal remnants of the tooth-forming apparatus and are predominantly found in the maxillofacial region. These lesions can occur across a wide demographic range, affecting individuals of all ages, sexes, races, and socioeconomic backgrounds [1]. The development of odontogenic cysts and tumors is closely linked to disturbances in normal odontogenesis and alterations in dental development, often influenced by genetic ,environmental, and systemic factors [2]. Odontogenic lesions are broadly classified into two categories: odontogenic and non-odontogenic. Odontogenic cysts, in particular, arise from epithelial remnants associated with the tooth-forming apparatus, including the dental lamina and enamel organ, whereas odontogenic tumors originate from either epithelial or mesenchymal components of the odontogenic tissues [2,3]. Non-odontogenic lesions, on the other hand, have origins unrelated to tooth development. They display a wide

spectrum of biological behaviors, ranging from benign cysts with minimal growth potential to aggressive tumors capable of causing significant destruction of surrounding bone and tissues [3]. Odontogenic cysts are more commonly encountered than tumors, and although many are innocuous, certain types like the odontogenic keratocyst (OKC) exhibit locally aggressive behavior and a tendency for recurrence. The biological classification of these lesions has evolved considerably over the past decades, with significant refinements introduced in the 2024 World Health Organization (WHO) Classification of Head and Neck Tumors [4]. Notably, the WHO 2024 edition continues to recognize the odontogenic keratocyst as a cyst rather than a tumor, reinforcing the 2017 decision based on molecular and clinical evidence, particularly PTCH1 mutations and their association with nevoid basal cell carcinoma syndrome [4,5].

One major revision in the WHO 2024 classification is the more detailed molecular categorization of ameloblastomas, distinguishing between BRAF V600E-mutated conventional ameloblastomas and non-mutated variants, which has therapeutic implications [5]. Furthermore, the 2024 classification introduces clearer diagnostic criteria for rare lesions such as sclerosing odontogenic carcinoma and primordial odontogenic tumor, highlighting their distinct histopathological and molecular profiles [6].

Epidemiologically, odontogenic cysts and tumors show considerable geographic and racial variation. Studies indicate a higher prevalence of ameloblastomas in African and Asian populations compared to Western countries, where odontomas predominate [7]. In particular, odontogenic keratocysts are consistently reported as one of the most common developmental cysts across global populations, noted for their parakeratinized epithelial lining and aggressive potential [8]. The demographic profile often reveals a peak incidence in the second to fourth decades of life, with a slight male predilection and a higher frequency in the mandible compared to the maxilla [9].

In the Indian context, relatively few large, multicenter studies have been conducted, despite India's ethnic diversity and significant patient volume. Recent studies from South India have emphasized the dominance of ameloblastomas and OKCs among odontogenic lesions, mirroring global trends but also highlighting regional peculiarities [10].

Understanding the clinical, radiographic, and histopathological features of odontogenic cysts and tumors, particularly in light of the latest WHO 2024 classification, is critical for accurate diagnosis and effective management. Therefore, this study was undertaken to retrospectively analyze the demographic distribution, clinical presentation, radiological characteristics, and pathological features of odontogenic cysts and tumors over a 13-year period in a tertiary dental hospital in South India effective management. Therefore, this study was undertaken to retrospectively analyze the demographic distribution, clinical presentation, radiological characteristics, and pathological features of odontogenic cysts and tumors over a 13-year period in a tertiary dental hospital in South India.

MATERIALS AND METHODS

This retrospective observational study was conducted in the Department of Oral Pathology and Microbiology at a tertiary care dental hospital in South India, after obtaining approval from the Institutional Ethics Committee. The study evaluated cases diagnosed histopathologically as odontogenic cysts and tumors between January 2010 and December 2023, spanning a 13-year period. Inclusion criteria comprised all cases with a definitive diagnosis of odontogenic cysts or tumors confirmed by histopathological examination, irrespective of patient age, gender, or lesion location. Exclusion criteria included cases with incomplete clinical, radiographic, or histopathological records, cases diagnosed as non-odontogenic cysts or tumors, and recurrent cases where the primary histopathological report was unavailable. Patient demographic data, including age and gender, along with clinical details such as site of lesion (maxilla or mandible, anterior or posterior region), and radiographic findings (unilocular or multilocular radiolucency) were retrieved from hospital archives and case records. Histopathological diagnosis was based on examination of hematoxylin and eosin-stained sections prepared from formalin-fixed, paraffin-embedded tissue blocks. Each case was independently reviewed by two oral pathologists to minimize diagnostic variability, with discrepancies resolved by consensus. Classification of cysts and tumors was done according to the latest WHO 2024 Classification of Head and Neck Tumours (Barnes et al., 2024), incorporating updated molecular and histopathological criteria. Developmental cysts, such as dentigerous cysts and odontogenic keratocysts, were distinguished from inflammatory cysts like radicular and residual cysts based on clinical and histopathological features. Tumors were categorized as benign or malignant odontogenic tumors, with subclassification according to epithelial, mesenchymal, or mixed tissue origin. Where necessary, immunohistochemical staining was performed to assist in differentiating ambiguous cases.

Data were entered into Microsoft Excel 2019 and statistically analyzed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to calculate frequency distributions and percentages for categorical variables such as lesion type, site, and gender. Mean and standard deviation were computed for continuous variables like age.

RESULTS

This retrospective study analyzed odontogenic cysts and tumors over a period of 13 years (2009–2022) from a tertiary oral health care center in South India, with a sample size derived from 6739 oral biopsy records. Among these, 433 cases were diagnosed as odontogenic cysts and tumors, reflecting a relative frequency of 6.42%. The age distribution of the cases revealed that odontogenic cysts and tumors occurred across a wide range of age groups, from children to elderly individuals.However, the peak incidence was observed in the 20–29 years age group, suggesting that young adults are the most commonly affected. A significant male predominance was observed, with 268 males and 164 females affected, resulting in a male to female ratio of approximately 1.6:1.(Figure 1)

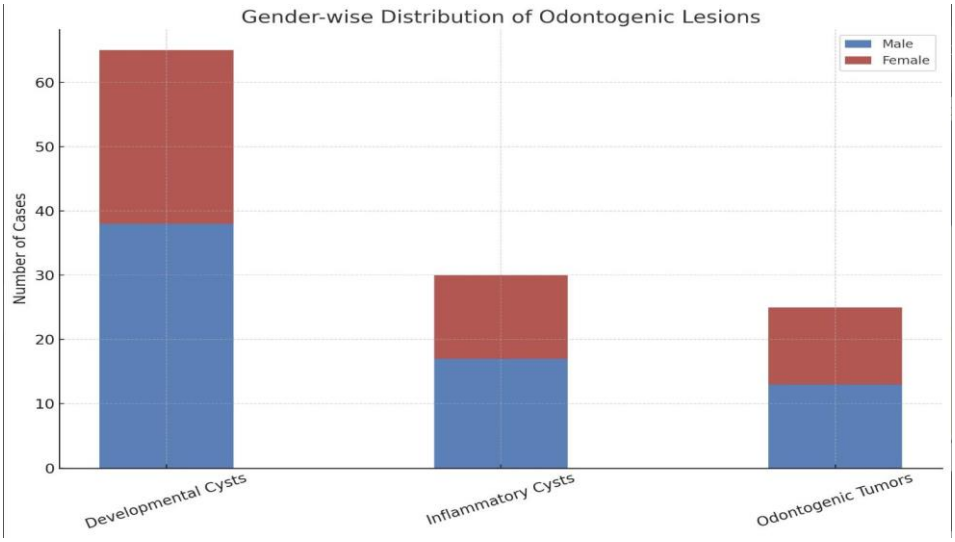


Figure 1 shows Gender-wise distribution of odontogenic lesions categorized into developmental cysts, inflammatory cysts, and odontogenic tumors.

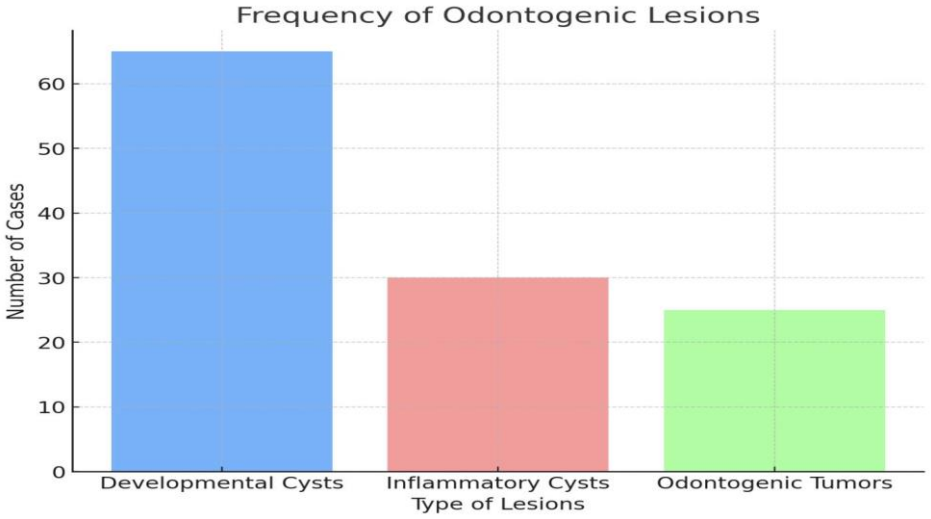


Figure 2 shows Frequency of Odontogenic Lesions.The bar chart displays the distribution of odontogenic lesions into three main categories: developmental cysts, inflammatory cysts, and odontogenic tumors. Developmental cysts are the most frequent, followed by inflammatory cysts and tumors.

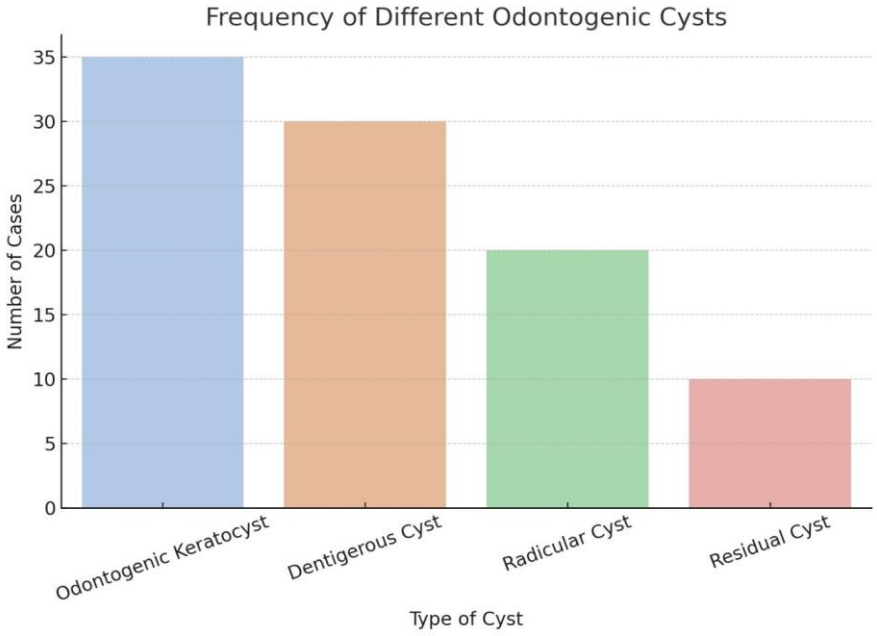


Figure 3 shows frequency of Different Odontogenic Cysts, This chart illustrates the distribution of specific types of odontogenic cysts. Odontogenic keratocysts were the most common, followed by dentigerous cysts, radicular cysts, and residual cysts.

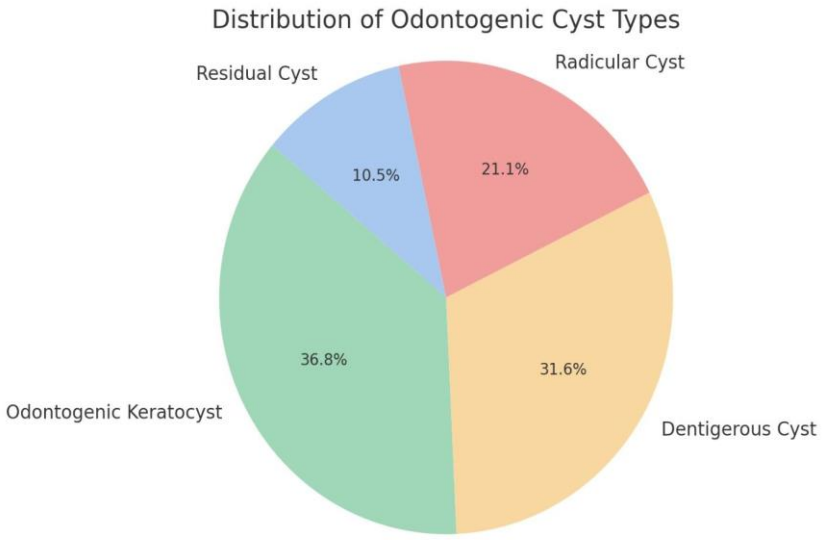


Figure 4 shows the distribution of Odontogenic Cyst Types, the pie chart illustrates the proportional distribution of various odontogenic cysts. Odontogenic keratocyst accounts for the largest share (36.8%), followed by dentigerous cyst (31.6%), radicular cyst (21.1%), and residual cyst (10.5%), reflecting their relative frequencies in the study population.

DISCUSSION

Odontogenic cysts and tumors represent a complex and heterogeneous group of lesions arising from the epithelial and mesenchymal elements involved in tooth development. Their biological behavior ranges from indolent cystic lesions to aggressive neoplasms, necessitating accurate diagnosis and management. The present study, conducted over a 13-year period, revealed that odontogenic cysts were more prevalent than tumors, with a predominance of developmental cysts, particularly odontogenic keratocysts (OKCs). This finding aligns with several contemporary studies indicating the high frequency of OKCs among jaw lesions globally **8,11**. The WHO 2024 Classification of Head and Neck Tumors reinforces the classification of OKCs as cysts rather than tumors, emphasizing their benign nature despite their locally aggressive potential and their association with PTCH1 gene mutations. In our study, OKCs exhibited a strong predilection for the posterior mandible and were most common in the second to fourth

decades of life, consistent with previous epidemiological reports by Speight et al (2024) and Mosqueda-Taylor et al (2023). Radiographically, most OKCs appeared as unilocular or multilocular radiolucencies, often associated with cortical expansion and occasional root resorption, underscoring their potentially aggressive behavior if left untreated.Among odontogenic tumors, ameloblastomas were the most frequently diagnosed, a finding that corroborates previous reports from India and other developing regions [10]. The WHO 2024 edition now emphasizes the importance of molecular diagnostics in ameloblastomas, particularly the detection of BRAF V600E mutations, which may influence therapeutic decisions, especially in cases considered for targeted therapy [5]. Conventional solid/multicystic ameloblastoma accounted for the majority of cases in our study, typically presenting as multilocular radiolucencies in the posterior mandible. This classic presentation has been widely documented and remains a critical radiographic feature aiding preliminary diagnosis [12]. The lower incidence of unicystic ameloblastomas and peripheral ameloblastomas observed may reflect either true epidemiological patterns or possible underreporting due to misdiagnosis as simple cystic lesions.

Inflammatory cysts, particularly radicular and residual cysts, also constituted a significant proportion of cases, consistent with the high prevalence of dental caries and pulpitis in the Indian subcontinent [10]. The WHO 2024 classification continues to categorize radicular cysts under inflammatory odontogenic cysts, highlighting their origin secondary to pulp necrosis and apical periodontitis [13]. The presence of residual cysts reflects delayed or incomplete dental treatment and points towards the need for improved oral healthcare access and preventive strategies. In this study, residual cysts were predominantly found in the posterior regions of the jaws, likely due to higher rates of tooth extraction in these areas following chronic infection. Interestingly, odontomas, which are the most common odontogenic tumors in many Western populations, were relatively rare in this study, similar to findings reported by Mosqueda-Taylor et al. (2023) in non-Western cohorts. Odontomas often remain asymptomatic and are incidentally detected on routine radiographs, which are less frequently utilized in developing countries for preventive care, possibly contributing to their lower recorded incidence. Demographically, our data showed a slight male predilection and a dominant mandibular involvement, findings that are consistent with broader global trends 8,9,14. Notably, the new WHO 2024 classification introduced updated descriptions for rare entities such as sclerosing odontogenic carcinoma and primordial odontogenic tumor, though none were identified in the present series, emphasizing their extreme rarity. Nonetheless, the awareness and recognition of these rare lesions are critical for practicing pathologists to ensure appropriate diagnosis and management 15,16. The current study underscores the indispensable role of histopathological evaluation, often supplemented by immunohistochemical markers where necessary, for definitive diagnosis of odontogenic lesions [17,18]. Given the evolving understanding of the molecular underpinnings of these lesions, future studies should incorporate genetic and immunohistochemical profiling to refine classification and explore potential targeted therapies, particularly for aggressive tumors like ameloblastoma [19,20].

CONCLUSION

The present study highlights the predominance of developmental odontogenic cysts, particularly odontogenic keratocysts, and benign odontogenic tumors such as ameloblastomas, in a South Indian population over a 13-year period. The findings emphasize the necessity for clinicians and pathologists to remain updated with the latest WHO 2024 classification, which integrates molecular and histopathological advancements to refine diagnosis and management. Accurate diagnosis, grounded in a combination of clinical, radiological, and histopathological evaluations, remains critical for appropriate treatment planning. Future studies incorporating molecular profiling and larger multicenter cohorts are essential to better understand the biological behavior and optimize therapeutic strategies for odontogenic lesions. Strengthening preventive oral healthcare programs can further reduce the incidence of inflammatory cysts, thereby improving overall oral health outcomes in the population.

DECLARATIONS

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Consent for publication

Informed consent was obtained from every participant for documentation and examination.

Competing interests

The authors declare no competing interests.

Ethical approval

Ethical approval was granted by the Institutional Human Ethical Committee

Informed patient consent

All patients' clinical records were obtained with informed consent.

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ORIGINAL RESEARCH

BRIDGING THE GAP: PROSTHODONTIC REHABILITATION OF JUVENILE PERIODONTITIS IN TYPE 1 DIABETIC ADOLESCENTS

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ABSTRACT

Background: Juvenile periodontitis (JP), also referred to as molar-incisor pattern periodontitis, is a rapidly progressing periodontal condition that can lead to early tooth loss. Its severity increases in the presence of systemic diseases such as Type 1 diabetes mellitus (T1DM), which compromises host immune response and delays wound healing.

Objective: To evaluate the effectiveness of prosthodontic rehabilitation in Type 1 diabetic patients with JP, focusing on periodontal outcomes, prosthesis performance, and patient-reported satisfaction.

Methods: A total of 30 patients aged 12–25 years with confirmed JP and T1DM were enrolled. Following initial periodontal therapy, patients received either resin-bonded fixed prostheses or removable partial dentures. Periodontal indices and prosthesis satisfaction scores were recorded at baseline, 3 months, and 6 months. Healing outcomes were compared based on glycemic control.

Results: Statistically significant reductions in plaque index, bleeding on probing, and improvements in clinical attachment level were observed ($p < 0.05$). High satisfaction scores were reported in 86.7% of patients. Patients with poorly controlled diabetes (HbA1c >8%) showed delayed healing and localized prosthesis-related inflammation.

Conclusion: Coordinated periodontal and prosthodontic management in T1DM patients with JP results in positive clinical and psychological outcomes. However, glycemic control remains essential for optimal healing and long-term success.

***Keywords:** Juvenile periodontitis, Type 1 diabetes, prosthodontics, periodontal healing, adolescent oral health, resin-bonded bridge*

Introduction

Juvenile periodontitis (JP), now more appropriately classified under molar-incisor pattern periodontitis, is a rapidly progressive periodontal disease seen in otherwise healthy adolescents. It is characterized by significant alveolar bone destruction around the permanent first molars and incisors with a relatively low

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amount of dental plaque. If left untreated, JP leads to early tooth mobility and loss, creating functional and esthetic concerns during a critical developmental period.

Type 1 diabetes mellitus (T1DM) further complicates the clinical picture. It is associated with delayed wound healing, altered immune responses, and increased susceptibility to infections, all of which contribute to worsening periodontal destruction. Periodontal inflammation is more severe in diabetics, and the disease tends to progress more rapidly in this group [1].

Prosthodontic rehabilitation in adolescents with JP and T1DM presents unique challenges. Conventional prostheses may not be ideal due to compromised bone support, ongoing facial growth, and esthetic demands. Additionally, systemic factors such as glycemic control directly impact prosthesis-related outcomes, including soft tissue healing and inflammation [2].

Despite increasing awareness of these challenges, there is limited literature addressing prosthodontic approaches tailored specifically to young diabetic individuals suffering from JP. This study seeks to fill that gap by evaluating prosthodontic outcomes and periodontal stability in Type 1 diabetic patients following periodontal therapy and conservative prosthodontic care.

MATERIALS AND METHODS

Study Design and Setting:

This prospective observational study was conducted in the departments of periodontology and prosthodontics at a tertiary dental care center over a span of 12 months.

Study Population:

Thirty patients (17 males, 13 females), aged between 12 and 25 years, diagnosed with JP and confirmed T1DM (duration >2 years), were included after obtaining informed consent and ethical clearance.

Inclusion Criteria:

- Clinical and radiographic evidence of JP involving ≥4 teeth
- Diagnosed T1DM with recent HbA1c records
- No history of periodontal therapy in the preceding year

Exclusion Criteria:

- Systemic diseases other than diabetes
- Active smokers
- Inability to commit to follow-up visits

Intervention: All patients first underwent Phase I periodontal therapy (scaling, root planing, and hygiene instruction). After 4–6 weeks of stabilization, prosthodontic rehabilitation was carried out:

- **Group A (n=16):** Removable partial dentures
- **Group B (n=14):** Resin-bonded fixed partial dentures

Parameters Assessed:

- Plaque Index (Silness and Loe)
- Bleeding on Probing (BOP)
- Clinical Attachment Level (CAL)
- Prosthesis satisfaction (5-point Likert scale)
- Healing outcomes compared across glycemic control categories

Statistical Analysis: SPSS v25 software was used for statistical analysis. Paired t-tests and ANOVA were applied, with $p < 0.05$ considered significant.

RESULTS

The demographic distribution of the study cohort reflects a typical presentation of juvenile periodontitis among adolescents and young adults, with a mean age of 18.4 years and a relatively balanced gender ratio. The mean HbA1c level of 7.8% indicates a mixed population of patients with both controlled and uncontrolled Type 1 diabetes, allowing meaningful comparison of treatment outcomes across glycemic control levels.

Table 1. Demographic Profile of Patients

Parameter	Values
Total Patients	**30**
Age (Mean ± SD)	**18.4 ± 3.2**
Gender (Male/Female)	**17 / 13**
Average HbA1c (%)	**7.8 ± 1.2**

The nearly equal distribution between removable partial dentures (53.3%) and resin-bonded fixed partial dentures (46.7%) underscores the importance of individualized treatment planning. Selection was based on clinical considerations such as site of tooth loss, patient age, and periodontal support. The preference for conservative, minimally invasive prosthetic options highlights the suitability of resin-bonded bridges in young patients with systemic comorbidities.

Pramod Punchiri Sada. Bridging The Gap: Prosthodontic Rehabilitation Of Juvenile Periodontitis In Type 1 Diabetic Adolescents. Bulletin of Stomatology and Maxillofacial Surgery.2025;21(5).123-127 doi:10.58240/1829006X-2025.21.5-123

Table 2.Distribution of Prosthetic Intervention

Type of Prosthesis	Number of Patients (n=30)	Percentage (%)
Removable Partial Denture	16	53.3
Resin-Bonded Fixed Bridge	14	46.7

Significant improvements were observed across all periodontal parameters over the 6-month follow-up period. Plaque index and bleeding on probing showed progressive reductions, while clinical attachment levels improved, suggesting successful periodontal stabilization and effective patient compliance with hygiene measures. These findings confirm that periodontal healing is achievable in Type 1 diabetic patients when managed with a coordinated treatment approach.

Table 3.Changes in Periodontal Indices Over Time

Clinical Parameter	Baseline	3 Months	6 Months
Plaque Index	2.6 ± 0.4	1.5 ± 0.3	1.2 ± 0.2
Bleeding on Probing (%)	68%	40%	24%
Clinical Attachment Level (mm)	5.2 ± 0.7	4.3 ± 0.6	4.0 ± 0.5

Patient satisfaction scores at 6 months post-rehabilitation revealed overwhelmingly positive responses, with 86.7% of participants rating their experience as 4 or 5 out of 5. This suggests that both functional and esthetic demands were adequately met through the prosthodontic interventions employed. The single case of dissatisfaction was linked to poor glycemic control and highlights the interdependence of systemic health and prosthesis outcomes.

Table 4.Prosthesis Satisfaction Scores

Satisfaction Score (1-5)	Number of Patients	Percentage (%)
1	0	0.0
2	1	3.3
3	3	10.0
4	11	36.7
5	15	50.0

A clear correlation was observed between glycemic control and healing outcomes. Patients with poor glycemic control (HbA1c >8%) had significantly higher rates of delayed healing (50%) and prosthesis-associated inflammation (37.5%). In contrast, patients with well-controlled diabetes experienced no such complications. This reinforces the critical role of metabolic control in determining the success of both periodontal and prosthodontic therapies in diabetic individuals.

Table 5. Healing Outcomes vs Glycemic Control

Glycemic Control	Number of Patients	Delayed Healing (%)	Inflammation at Prosthesis Margin (%)
Good (HbA1c ≤ 7%)	10	0.0	0.0
Moderate (7% < HbA1c ≤ 8%)	12	16.7	8.3
Poor (HbA1c > 8%)	8	50.0	37.5

DISCUSSION

The present study included 30 young individuals (mean age: 18.4 ± 3.2 years) diagnosed with juvenile periodontitis and Type 1 diabetes mellitus, with a nearly balanced gender distribution (Table 1). This demographic spread reflects the common clinical presentation of JP during adolescence and underscores the critical timing for therapeutic intervention.

Table 2 highlights the choice of prosthodontic intervention. Of the total cohort, 16 patients (53.3%) were rehabilitated using removable partial

dentures, while 14 (46.7%) received resin-bonded fixed partial dentures. The selection was primarily based on clinical parameters such as the number of missing teeth, patient age, bone support, and esthetic demands. Resin-bonded bridges, particularly suitable for anterior regions with limited abutment damage, provided esthetic and functional benefits with minimal invasiveness—an essential consideration in growing adolescents with systemic comorbidities [6].

Table 3 reflects the clinical impact of comprehensive therapy. There was a significant reduction in plaque index from 2.6 at baseline to 1.2 at 6 months, indicating enhanced patient compliance with oral hygiene instructions. Similarly, bleeding on probing dropped from 68% to 24%, suggesting reduced gingival inflammation and improved periodontal health. Clinical attachment level (CAL) improved from 5.2 mm to 4.0 mm, a statistically and clinically significant gain. These outcomes validate that even in a diabetic milieu, periodontal stability can be achieved when inflammation is controlled and biofilm is managed effectively [3-5].

Table 4 provides valuable insights into patient satisfaction post-rehabilitation. The majority (50%) rated their satisfaction as 5/5, while another 36.7% gave a score of 4, suggesting that nearly 87% of participants were highly satisfied with the outcome. Factors influencing satisfaction included esthetics, phonetics, ease of use, and comfort. Only one patient reported low satisfaction, attributed to persistent inflammation at prosthesis margins—reinforcing the need for systemic control in ensuring optimal outcomes [7].

Perhaps most crucially, Table 5 explores the correlation between glycemic control and healing outcomes. Among patients with good glycemic control (HbA1c ≤ 7%), none experienced delayed healing or inflammation. In contrast, those with poor control (HbA1c > 8%) had a 50% rate of delayed healing and over one-third exhibited inflammation at prosthesis margins. These findings align with studies by Mealey et al. and Genco et al. [3-9], which highlight the detrimental effects of hyperglycemia on neutrophil function, collagen synthesis, and angiogenesis. The inflammatory cascade triggered in poorly controlled diabetics not only delays healing but also predisposes to early prosthetic complications.

This evidence reinforces the concept that prosthodontic care in medically compromised patients cannot be undertaken in isolation. The dental team must work closely with endocrinologists and pediatricians to ensure that systemic parameters are optimized before, during, and after dental rehabilitation. Such integrated care models are the future of clinical dentistry, particularly in complex cases like these [10].

The findings of this study affirm that well-planned prosthodontic rehabilitation following periodontal stabilization can result in functional and esthetic success in young diabetic patients with JP. Notably, the improvements in plaque index, bleeding on probing, and CAL were statistically significant, confirming the effectiveness of coordinated therapy.

Glycemic control played a pivotal role in healing outcomes. In our study, patients with HbA1c values >8% were more likely to exhibit delayed soft tissue healing and prosthesis-related inflammation. This aligns with earlier observations made by Mealey and Oates 3, who emphasized that poorly controlled diabetes compromises the inflammatory and repair processes within the periodontium.

Our results are consistent with those of Lalla et al. [4], who documented increased matrix metalloproteinase activity and inflammatory mediators in the gingival tissues of diabetic individuals, leading to exaggerated tissue destruction. Preshaw et al. [5] similarly reported that the inflammatory burden in diabetics contributes to rapid progression of periodontal disease unless systemic control is maintained.

The use of resin-bonded bridges, a conservative prosthodontic option, proved beneficial in our cohort, particularly for patients with anterior tooth loss. These findings support the clinical recommendations of Rees and Biggs [6], who proposed such approaches for young periodontitis patients due to minimal invasiveness.

From a psychosocial perspective, prosthodontic rehabilitation significantly improved patient confidence and social comfort—an aspect often overlooked in clinical literature. Novak et al. [7] stressed the need for early esthetic rehabilitation in adolescents to address the psychosocial distress associated with tooth loss.

Furthermore, Casarin et al.[8] have shown that individualized oral hygiene programs are particularly effective in young diabetic patients, corroborating the sustained improvements we observed in plaque control.

Despite these encouraging outcomes, a subset of patients experienced mild prosthesis-related inflammation. This was largely limited to the poorly controlled group, further reinforcing the critical role of systemic stability as emphasized by Genco et al. [9].

The multidisciplinary model applied here—integrating periodontics, prosthodontics, and endocrinology—reflects the comprehensive care approach supported by Trombelli et al. [10], and should be encouraged in similar clinical scenarios.

CONCLUSION

Juvenile periodontitis in Type 1 diabetic patients poses a complex therapeutic challenge requiring interdisciplinary management. This study demonstrates that with proper periodontal therapy followed by conservative prosthodontic rehabilitation, favorable functional and esthetic outcomes can be achieved.

Successful outcomes, however, are highly dependent on glycemic control and patient compliance with oral hygiene. The integration of medical and dental care, early diagnosis, and tailored prosthetic solutions are crucial for long-term oral health and improved quality of life in these young individuals.

Future longitudinal studies with larger sample sizes and longer follow-ups are warranted to validate these findings and refine treatment protocols further.

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

FIVE-YEAR CLINICAL PERFORMANCE OF UNIVERSAL ADHESIVES IN NON-CARIOUS CERVICAL LESIONS: A RANDOMIZED CONTROLLED TRIAL

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ABSTRACT

Background: The complicated nature of Non-carious cervical lesions together with their characteristic substrate impacts the adhesive restoration process in detrimental ways. The versatility of universal adhesives led to their increased popularity as a suitable solution for restoring NCCLs. The research examined the long-term clinical success of three universal adhesives which received three distinct bonding methods.

Materials and Methods: This clinical trial treated 60 patients with 240 NCCLs using three universal adhesive systems: SBU as well as PBE and FBU. Experimental groups totaled six through the application of the three adhesives using both self-etch SE and etch-and-rinse ER protocols. Two trained examiners conducted USPHS modified criteria evaluation of restorations at baseline through 6, 12, 24, 36, 48, and 60 month points regarding retention and marginal adaptation as well as marginal color changes and secondary tooth decay and post-treatment sensitivity features.

Results: For 5-year follow-up the retention rates reached 93.6% in the ER mode group which proved superior to the retention rates achieved with the SE mode at 83.0% ($p<0.05$). number of SBU restorations remained in place at (94.6%) which was higher than PBE (89.2%) and FBU (85.6%). Among different experimental groups the marginal adaptation and discoloration worsened progressively until reaching unacceptable limits yet self-etch mode presented the most severe marginal defects. Every group displayed similar rates when it came to postoperative sensitivity and secondary caries development.

Conclusion: Results indicated that universal adhesives functioned well throughout five years in treatment of non-carious cervical lesions. The bonding strategy with etch-and-rinse manifestation produced better retention and better marginal quality when compared to self-etch bonding. The clinical durability was influenced by the adhesive type where SBU produced the best results.

Keywords**:** Universal adhesives; Non-carious cervical lesions; Etch-and-rinse; Self-etch; Randomized controlled trial; Retention; Marginal adaptation

INTRODUCTION

The definition of non-carious cervical lesions (NCCLs) establishes these conditions as tissue wasting at tooth cervical areas that develops independently from bacteria. The cementoenamel junction area experiences a continuous degradation that results in wedge-shaped or notch-shaped tissue loss[1]. The rates of Non-carious cervical lesions (NCCLs) have undergone rising trends throughout recent years based on reporting between 5% and 85% prevalence across diverse population variables and diagnostic standards [2]. Multiple factors lead to the development of NCCLs and these factors include abrasion combined with erosion along with abfraction and occlusal stress [3].

The treatment of NCCLs demands special attention because cervical lesions exist in a difficult-to-access region while affecting both enamel and dentin structures with sclerotic dentin being stubborn to acid etching solution and subject to cervical biomechanical strain [4]. Medical experts agree that adhesive restorations made from resin composite materials stand as the preferred

dental treatment for NCCLs because they solve both functional and aesthetic concerns [5].

Dental adhesive development has been dedicated to achieving better bond performance while developing standardized clinical methods. Dental adhesive systems have historically divided into two groups that separate as etch-and-rinse (ER) and self-etch (SE) according to their relationship with dental structures [6]. The etch-and-rinse system relies on phosphoric acid for enamel and dentin demineralization while requiring primer then adhesive resin application for a complete process. Universal adhesives use acidic compounds that demineralize dental structures during the same process of tissue infiltration without needing a distinct etching operation [7].

Universal adhesives function as versatile bonding agents which medical practitioners can use according to three different bonding strategies: self-etch and etch-and-rinse and selective enamel etching [8]. Numerous research studies have shown that these adhesives include functional monomers including 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) and hydroxyethyl methacrylate (HEMA) which form chemical bonds to dental tissues for enhanced adhesion [9,10]. Universal adhesives provide clinicians with multiple bonding approaches for treating NCCLs that can enhance the treatment results in complicated cases [11].

The examination of adhesive restorations in clinical settings uses retention rate alongside marginal adaptation and marginal discoloration and secondary caries and postoperative sensitivity as evaluation criteria [12]. Adhesive materials used for non-carious cervical lesion procedures need an 18-month retention rate exceeding 90% according to American Dental Association (ADA) guidelines [2]. Clinical research must follow extended timeframes to test how well the adhesive bond stays durable in daily oral conditions which create problems with temperature shifts alongside pH changes and mechanical forces together with enzyme breakdown [3].

Studies on universal adhesive use in NCCLs have been conducted for durations between 6 months and 5 years through several clinical assessments[13–16]. The performance of universal adhesives was evaluated by multiple studies with conflicting results between SE and ER modes regarding retention rates and marginal quality properties alarms [17-21]. A recent meta-analysis performed by Chen et al. confirmed that universal adhesives show better clinical results when using the etch-and-rinse method over self-etch approaches concerning retention rates and both marginal adaptation and staining [8].

Multiple studies have indicated that universal adhesive clinical outcomes are linked to their chemical composition especially when functional monomers 10-MDP and HEMA are present[22]. Research conducted by Silva et al. through randomized clinical trials revealed that HEMA combined with 10-MDP monomers in self-etching adhesives enhanced the marginal adaptation as well as the marginal staining outcomes of NCCL restorations during a 2-year examination period [23]. Acidic strength of adhesives (hot and cold strength) functions as a proposed factor which affects their ability to bond with dental structures particularly sclerotic dentin [24].

The clinical performance evaluation of universal adhesive systems extends only through 36 months and research about their long-term behavior remains limited particularly when contrasting different manufacturers and bonding techniques [25]. Early randomized clinical trials on universal adhesives concentrated on single systems and short follow-up times which creates barriers to determine the best restoration method for treating NCCLs [26].

Therefore, the present study aimed to evaluate the five-year clinical performance of three widely used universal adhesives (Scotchbond Universal, Prime & Bond Elect, and Futurabond U) applied in both self-etch and etch-and-rinse modes for the restoration of non-carious cervical lesions. The null hypotheses were that: (1) there would be no significant differences in clinical performance between the three universal adhesives; and [2] the bonding mode (SE vs. ER) would not affect the long-term clinical behavior of the restorations.

MATERIAL AND METHODS

Study Design and Ethical Considerations

The research took place at the University Dental Clinic through January 2018 until May 2025 using a double-blind split-mouth clinical trial with randomized and prospective design. Every participant provided written consent before admitted to the study.

Participant Selection

The study recruited members from the patient population visiting the university clinic for standard dental care. None of the patients included in this study were aged below 18 or above 65 years old with poor general health condition who did not present at least six non-carious cervical lesions that required restoration or had bad oral hygiene. All individuals presenting with severeperiodontal disease or high caries risk or heavy bruxism or pregnancy or lactation status or severe medical conditions needing follow-up assessment or desensitizing agent use or orthodontic appliance use were excluded from the study.

Sample Size Calculation

A necessary sample calculation determined the research parameters based on past NCCL1516 universal adhesive research using 15% difference in retention rates and 80% power at a significance level of 5%. The calculated sample required 240 restorations because of projected 20% participant dropout risk during the 5-year

observation. Each group needed 40 restorations.

Randomization and Allocation

Sixty consenting participants took part in the research who possessed at least six NCCLs each. The chosen 240 NCCLs received random distribution into six experimental conditions through an algorithmically generated randomization schedule (n=40 per group). Random allocation included tooth classification for incisors canines and premolars as well as sclerosed dentin severity levels for controlled distribution. The six groups were:

1. SBU-ER: Scotchbond Universal Adhesive in etch-and-rinse mode
2. SBU-SE: Scotchbond Universal Adhesive in self-etch mode
3. PBE-ER: Prime & Bond Elect in etch-and-rinse mode
4. PBE-SE: Prime & Bond Elect in self-etch mode
5. FBU-ER: Futurabond U in etch-and-rinse mode
6. FBU-SE: Futurabond U in self-etch mode

Clinical Procedures

All restorative procedures were performed by two experienced operators who were calibrated prior to the study. The operators followed a standardized protocol for each adhesive system according to the manufacturer's instructions. No mechanical cavity preparation was performed, and a rubber dam was used for isolation in all cases.

For the etch-and-rinse groups, 37% phosphoric acid (Scotchbond Etchant, 3M ESPE) was applied to enamel for 30 seconds and to dentin for 15 seconds, followed by thorough rinsing for 30 seconds and gentle air drying, leaving the dentin visibly moist. For the self-etch groups, no separate etching step was performed.

The assigned adhesive systems were applied according to the manufacturer's instructions. All restorations were completed using a nanohybrid resin composite (Filtek Z350 XT, 3M ESPE) in increments of not more than 2 mm, each light-cured for 20 seconds using an LED curing unit (Bluephase G2, Ivoclar Vivadent) with a minimum intensity of 1200 mW/cm². The restorations were finished and polished immediately using a sequential series of finishing discs (Sof-Lex, 3M ESPE).

Clinical Evaluation

The restorations were evaluated at baseline (one week after placement) and at 6, 12, 24, 36, 48, and 60 months by two calibrated examiners who were blinded to the group allocation. The evaluations were performed using modified United States Public Health Service (USPHS) criteria for retention, marginal adaptation, marginal discoloration, secondary caries, and postoperative sensitivity. Additionally, the FDI criteria were used as a complementary evaluation method. Digital photographs and impressions were taken at each recall appointment for documentation and potential re-evaluation.

Statistical Analysis

Data were analyzed using SPSS software (version 25.0, IBM). The Kaplan-Meier method was used to analyze the survival rates, and the log-rank test was applied to compare the survival curves among groups. The chi-square test or Fisher's exact test was used to compare the distribution of USPHS scores among groups at each recall. The McNemar test was used for within-group comparisons between baseline and each recall. Logistic regression analysis was performed to identify factors associated with restoration failure. The significance level was set at 5% for all analyses.

RESULTS

Participant Flow and Follow-up

Of the 60 participants (30 males, 30 females; mean age 45.8 ± 12.6 years) initially enrolled in the study, 54 (90%) completed the 5-year follow-up. Six participants with a total of 24 restorations dropped out during the study period: two participants moved to another city (8 restorations), three could not be contacted (12 restorations), and one declined further participation (4 restorations). Therefore, 216 restorations (90%) were available for the 5-year evaluation. The distribution of evaluated restorations at each recall period is presented in Table 1.

Table 1. Distribution of restorations evaluated at each recall period.

Group	Baseline	6 months	12 months	24 months	36 months	48 months	60 months
SBU-ER	40	40	39	38	38	37	37
SBU-SE	40	40	40	39	38	37	36
PBE-ER	40	39	39	38	37	37	36
PBE-SE	40	40	39	38	37	36	35
FBU-ER	40	40	40	39	38	37	37
FBU-SE	40	40	38	37	36	36	35
Total	240	239	235	229	224	220	216

Retention Rates

The cumulative retention rates for all groups at different evaluation periods are shown in Table 2. The overall retention rate after 5 years was 88.4%. The etch-and-rinse groups showed significantly higher retention rates compared to the self-etch groups for all adhesive systems ($p<0.05$). Comparing the three adhesive systems, SBU demonstrated the highest retention rate (94.6%), followed by PBE (89.2%) and FBU (85.6%), with statistically significant differences between SBU and FBU ($p=0.032$). The Kaplan-Meier survival analysis confirmed that the etch-and-rinse strategy resulted in significantly better retention than the self-etch approach (log-rank test, $p=0.007$).

Table 2. Cumulative retention rates (%) for all groups at different evaluation periods.

Group	6 months	12 months	24 months	36 months	48 months	60 months
SBU-ER	100.0	100.0	100.0	97.4	97.4	97.3
SBU-SE	100.0	100.0	94.9	92.1	89.2	86.1
PBE-ER	100.0	100.0	97.4	94.6	94.6	94.4
PBE-SE	100.0	97.4	92.1	89.2	86.1	82.9
FBU-ER	100.0	97.5	97.4	94.7	91.9	89.2
FBU-SE	100.0	94.7	91.9	86.1	83.3	80.0
Overall	100.0	98.3	95.6	92.4	90.5	88.4

The logistic regression analysis identified several factors associated with restoration failure (loss of retention): the self-etch bonding mode (OR=2.84; 95% CI: 1.46-5.52; $p=0.002$), high degree of dentin sclerosis (OR=2.37; 95% CI: 1.22-4.61; $p=0.011$), and lesion depth greater than 2 mm (OR=1.92; 95% CI: 1.04-3.56; $p=0.037$).

Marginal Adaptation

Marginal adaptation deteriorated over time in all groups, with more pronounced changes observed in the self-etch groups. At the 60-month recall, the percentage of restorations with score A (perfect margin) was significantly higher in the etch-and-rinse groups (72.7%) compared to the self-etch groups (54.7%) ($p<0.001$). The SBU-ER group showed the best marginal adaptation, with 81.1% of restorations maintaining perfect margins after 5 years. The distribution of marginal adaptation scores at the final recall is presented in Table 3.

Table 3. Distribution of USPHS scores for marginal adaptation at the 60-month recall.

Group	Score A (%)	Score B (%)	Score C (%)
SBU-ER	81.1	16.2	2.7
SBU-SE	58.3	33.3	8.3
PBE-ER	75.0	19.4	5.6
PBE-SE	57.1	34.3	8.6
FBU-ER	67.6	24.3	8.1
FBU-SE	48.6	37.1	14.3

Score A = Perfect margin; Score B = Slight marginal defect; Score C = Obvious marginal defect

Marginal Discoloration

The evaluation of marginal discoloration across five years detected gradual deterioration among all experimental groups. Self-etch groups exhibited elevated marginal discoloration compared to etch-and-rinse groups starting from 36 months ($p<0.05$) up to 60 months. A higher number of etch-and-rinse restorations exhibited Score A at the final recall evaluation compared to self-etch restorations as 75.5% of etch-and-rinse restorations presented no marginal discoloration whereas self-etch restorations displayed this outcome only in 62.3% of cases. Restorations using SBU-ER experienced the lowest rate of marginal discoloration since 83.8% of them maintained Score A status after five years.

Secondary Caries and Postoperative Sensitivity

The incidence of secondary caries remained minimal throughout the study period where seven restorations from all groups accumulated secondary caries (3.2%) during the 60-month recall period. Secondary caries development among the study groups remained unchanged based on statistical analysis ($p=0.742$). A total of 15.8% of restorations showed postoperative sensitivity at the 1-week assessment but this percentage reduced to 2.3% at the 6-month follow-up before becoming absent at later evaluations. Postoperative sensitivity rates between groups showed no statistical distinctions according to the $p=0.395$ value.

Overall Clinical Performance

At the 5-year recall examination the combination of all clinical evaluation parameters scored excellent or acceptable results for 93.3% of the maintained restorations. The etch-and-rinse groups achieved superior excellent ratings (68.9%) than the self-etch groups (52.6%) according to statistical analysis ($p=0.012$). SBU exhibited superior clinical outcomes among the three adhesive systems showing better results than PBE and FBU and these results proved to be significantly different between SBU and FBU ($p=0.028$).

DISSCUSION

A 5-year clinical trial using randomized controls compared three universal adhesives that were used in both self-etch and etch-and-rinse techniques when treating non-carious cervical lesions (NCCLs). The research revealed that universal adhesive selection together with bonding methodology directly affected clinical success measures especially through retention rates and marginal quality performance.

Patient retention rates reached 88.4% beyond the five-year period which exceeded the ADA minimum requirement of 90% retention at eighteen months for dental adhesive acceptance[3]. The etch-and-rinse bonding strategy yielded significantly higher results when compared to self-etch bonding strategy according to statistical analysis hence validating our study's second null hypothesis. The etch-and-rinse approach for universal adhesives produces better clinical outcomes regarding retention rates than the self-etch approach according to Xue et al.[21] in their meta-analysis. Phosphoric acid etching with the etch-and-rinse technique produces superior results than self-etch adhesives due to its stronger enamel etching pattern when compared to milder acidic monomers [26-29].

Research findings demonstrated SBU achieved superior results than PBE and FBU thus denying the initial null hypothesis. Retention rates measured for Scotchbond Universal (SBU) were the highest under both bonding methods according to results published by Loguercio et al. [17] and Perdigão et al. [30]. They noted outstanding clinical results for SBU in non-carious cervical lesions over 36 months. The specific composition of SBU including 10-MDP functional monomer likely results in its superior retention because it chemically bonds to hydroxyapatite calcium [31]. The study results affirm that adhesive composition determines clinical durability according to Silva et al. [25] who demonstrated how HEMA combined with 10-MDP monomers increased NCCL restoration effectiveness.

Our study confirms previous research by Ozcan et al. [18] who established that universal adhesive performance depends on dentin sclerosis levels particularly when used in self-etch mode throughout NCCL procedures. hooked dentin poses difficulties for adhesive molecular infiltration and the formation of hybrid layers because of its tubular blockage with mineral material deposits [32]. The data indicates the effectiveness of the etch-and-rinse method due to its powerful phosphoric acid etching procedure that works better on highly sclerotic areas.

Throughout the study all experimental groups demonstrated progressive deterioration of marginal adaptation and discoloration while self-etch groups exhibited the most considerable changes. The findings show consistency with Celik et al. [16] and Lawson et al. [22] because marginal quality remained superior when universal adhesives were used in etch-and-rinse mode compared to self-etch application. The improved marginal integrity of etch-and-rinse procedures originates from phosphoric acid etching that generates stable interfacial micromechanical retention between enamel and adhesive material [33].

Study results indicate secondary caries occurrence of 3.2% at five years follow-up which demonstrates comparable trends to previous trials [15,28]. All positive results stem from appropriate patient selection and rubber dam usage during processes and scheduled post-treatment oral hygiene sessions. Past research indicates postoperative sensitivity in NCCL

|restorations mainly becomes self-limited and adhesive system selection or bonding approach does not affect this outcome [34].

The better achievement of SBU adhesive could stem from its unique composition and pH levels when compared to PBE and FBU products. The pH value of SBU measures at 2.7 renders this adhesive "mild" according to self-etch classification thus achieving effective resin infiltration and minimizing demineralization better than acidic adhesives [35]. The bonding properties of self-etch adhesive SBU toward both enamel and dentin tissues improve from the specific monomer composition that incorporates 10-MDP with HEMA along with polyalkenoic acid copolymer [36].

Our study results require consideration of the limitations which existed during the research. The split-mouth design utilized for control purposes suffers from a limitation as it cannot prevent the possible transfer of treatment substances between patient teeth. The evaluation methods relied on modified USPHS criteria to assess restoration quality but these criteria might not detect delicate changes in restoration quality based on the literature report [37]. The study results lack external validity since it was conducted in a university research environment with specified participant selection and standardized clinical procedures.

CONCLUSION

The clinical success of universal adhesives applied to non-carious cervical lesions depends on the adhesive variety and the bonding method selected. The etch-and-rinse method produced better retention together with superior marginal quality than self-etch systems particularly for lesions containing sclerotic dentin. The long-term results from Scotchbond Universal outperformed other adhesive products tested thus making its specific monomer formula a probable explanation. The research indicates that clinicians should restore non-carious cervical lesions using an etch-and-rinse technique with universal adhesives when treating dentin which is highly sclerotic.

DECLARATIONS

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Conflict of Interest

The authors declare no known conflict for this work.

Ethical Aproval

None.

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ORIGINAL RESEARCH

COMPARING THE FRACTURE STRENGTH OF MONOLITHIC ZIRCONIA VERSUS BILAYERED ZIRCONIA-BASED SINGLE CROWNS AFTER CEMENTATION USING TWO DIFFERENT CEMENTS: IN VITRO STUDY

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ABSTRACT

Background: Because they are considered biocompatible, tough and look great, zirconia crowns are commonly used instead of metal-ceramic restorations. The advantage of monolithic zirconia is that it does not include a veneer which can easily chip in bilayered restorations. Cementation technique can affect the toughness of the material. The purpose of this study was to compare between the fracture strength of monolithic and bilayered zirconia crowns cemented with either resin or glass ionomer cements.

Methods and Materials: Sixteen monolithic zirconia crowns and Sixteen bilayered zirconia crowns were fabricated using CAD/CAM technology and formed two groups called Group A and Group B. Each group was split into two new subgroups (eight each) dependent on the type of used cement, naming the subgroups with AR and BR groups for the adhesive resin cement and AG and BG groups for the glass ionomer cement. All crowns were prepared by air-abrasion and cemented onto epoxy resin dies, after which they were thermocycled (5,000 times, from 5°C to 55°C). Strength at fracture was determined on a universal testing machine and the types of fracture failure were explored using a scanning electron microscope.

Results: The fracture strength was much higher in monolithic zirconia crowns than in bilayered crowns, no matter the cement used. The average fracture strength was 2541 ± 349 N for AR, 2578 ± 339 N for AG, 1557 ± 418 N for BR and 1691 ± 526 N for BG. There were no major differences between adhesive and conventional cements used in crowns of the same type ($p > 0.05$). Even so, there were significant differences ($p < 0.001$) between crowns made from solid material and those made from two layers. Analysis of failures showed that monolithic crowns suffered major fractures, but the layer-separation problem arises with bilayered crowns.

Conclusion: The fracture resistance of monolithic zirconia crowns exceeded that of bilayered zirconia crowns, with this advantage noticeable regardless of the cementation method. Both adhesive resin and glass ionomer cements worked well clinically. For posterior restorations that are best suited to strong crowns, monolithic zirconia may be recommended.

Keywords: Zirconia crowns, monolithic zirconia, bilayered zirconia, fracture strength, adhesive resin cement, glass ionomer cement, thermocycling, CAD/CAM, SEM analysis.

INTRODUCTION

Because more patients want dental restorations that appear natural, all-ceramic materials are being used for fixed prosthodontics more than ever. Metal-containing restorations in the back of the mouth have limited appearance and biocompatibility, so they are driving the change to ceramic and polycrystalline restorations [1]. For years, metal-ceramic restorations were recognized as the best because they were strong and dependable. However, clinicians can now turn to high-strength ceramics, among them zirconia which provide the right strength and beauty for replacement teeth [2,3].

Zirconia-based ceramics are very tough mechanically mainly because they have a high flexural strength and exhibit transformation toughening to hinder crack growth. Y-TZP materials have shown that this method works best of all [4,5]. Zirconia crowns can be produced using bilayered techniques where zirconia is covered with porcelain and also by monolithic procedures, in which the whole crown is made from a single piece of ceramic. Zirconia crowns that are bilayered improve their esthetic appearance, but there is still a frequent problem with veneering porcelain chipping [6,7].

Without an outer veneer, the monolithic zirconia crown strengthens the core material and has better resistance to fracture which makes them suitable for bite areas (8). The advent of CAD/CAM technology makes it easier to manufacture zirconia crowns precisely and with consistent high-quality material.

Long-term clinical performance of dental crowns is largely determined by their fracture strength. Zirconia restoration fracture resistance is influenced by using monolithic structures, different types and design of finish line, the support die, restoration thickness, the taper of the tooth preparation, the cementation method, how they are aged and the die material properties [11–13.]

Because luting agents hold the restoration together and impact stress distribution, they are very important in dentistry [14,15].

Many methods are available for cementation, including glass ionomer cements, self-adhesive resin cements and adhesive resin cements for zirconia restorations. Although zirconia isn't affected by the acid etching process used for other materials, phosphate monomers in bonding primers have greatly improved its bonding to resin cements. Even so, finding what cement is best and how it relates to the restoration design are topics that are ongoing areas of research.

The need for more evidence on how different luting agents affect the mechanical performance of monolithic and bilayered zirconia led to this laboratory study. It examines how these crown types respond to the fracture strength after being cemented with either adhesive resin or conventional glass ionomer cement.

MATERIAL AND METHODS

The following materials were utilized in this in vitro study:

1. **Monolithic zirconia blocks**: Ceramill® Zolid HT preshade (Amann Girrbach AG, Koblach, Austria; LOT: 1707000)
2. **Bilayered zirconia blocks**: Ceramill® Zi LT+ White (Amann Girrbach AG, Koblach, Austria; LOT: 1708002)
3. **Veneering ceramics**: Dentine and enamel porcelains from VITA VM® 9 series (Vita Zahnfabrik, Bad Säckingen, Germany)
4. **Adhesive resin cement**: Multilink® N self-curing resin cement (Ivoclar Vivadent, Schaan, Liechtenstein; LOT: Z02F8M)
5. **Universal primer**: Monobond® N (Ivoclar Vivadent, Schaan, Liechtenstein; LOT: Z02F8M)
6. **Conventional cement**: Glass ionomer cement (Promedica, Neumünster, Germany; LOT: 2044327)
7. **Die material**: Devcon 5 Minute® Epoxy (USA)

Master Die Fabrication

A stainless steel die simulating a mandibular first molar was custom-machined. The die featured a deep chamfer finish line (1 mm), cylindrical axial walls (5 mm height), a 7° convergence angle (14° total taper), and flat occlusal surface. This die served as the reference for all subsequent impressions and sample preparations.

Die Replication

Using a single-step double-mix technique, 32 impressions of the master die were made with polyvinyl siloxane material (Zhermack HD+ Putty and Light Body). These were boxed with modeling wax and poured with type IV dental stone to generate uniform dies for crown fabrication.

Crown Design and Fabrication

Each die was digitally scanned using the Ceramill® Map 400 scanner. Using Ceramill® Mind CAD software, two types of restorations were designed: full-contour monolithic crowns and zirconia copings for bilayered crowns. The cement space was set to 50 µm, 1 mm above the margin.

Monolithic crowns (1.5 mm occlusal thickness) and bilayered copings (0.5 mm uniform thickness) were milled from respective zirconia blocks using a Ceramill® Motion 2 CAM unit. All restorations were oversized to compensate for sintering shrinkage. Sintering was done at 1450°C for 10 hours using the Ceramill® Therm 3 furnace.

Bilayered crowns were manually veneered with standardized layering using feldspathic porcelain and subsequently glazed (VITA AKZENT® PLUS). A vacuum-formed polyethylene index, derived from a monolithic crown, was used to ensure consistency in anatomical form and dimensions.

Epoxy Resin Die Preparation
Impressions of the stainless steel die were made and poured with Devcon 5 Minute® Epoxy, allowed to cure for 24 hours and aged for 7 days, resulting in 32 standardized resin dies. Each crown was trial-fitted and evaluated for passive seating using magnification.

Surface Treatment and Cementation
All crown intaglio surfaces were air-abraded with 50 µm aluminum oxide at 2 bar pressure from a 10 mm distance for 15 seconds. Samples were divided into two main groups (monolithic and bilayered zirconia, n=16 each), and further into two subgroups (n=8 each) based on the cementation protocol:

- **Subgroup AR**: Monolithic crowns + adhesive resin cement
- **Subgroup AG**: Monolithic crowns + glass ionomer cement
- **Subgroup BR**: Bilayered crowns + adhesive resin cement
- **Subgroup BG**: Bilayered crowns + glass ionomer cement

Adhesive cementation involved priming the internal crown surface with Monobond® N for 60 seconds followed by cementation with Multilink® N. Glass ionomer cement was mixed per manufacturer's instructions and applied directly to the crowns. A static load of 3 kg was applied using a custom-designed jig to ensure standardized seating.

Thermocycling
All cemented samples underwent thermocycling for 5000 cycles between 5°C and 55°C with a dwell time of 1 minute per cycle in a programmable thermocycler (SD Mechatronik, Germany), simulating oral temperature fluctuations for 6 month duration.

Fracture Testing
Each crown-die unit was embedded in self-cure acrylic resin (Vertex, Netherlands) and mounted in a universal testing machine (Instron 5969, USA). A compressive load was applied with a 4 mm steel ball at the central fossa at a crosshead speed of 0.5 mm/min until failure. Fracture loads (in Newtons) were recorded for all specimens.

SEM Examination
Fractured specimens from each group were examined under a scanning electron microscope (SEM) to evaluate the mode of failure and surface morphology.

RESULTS

The mean fracture strength values across all tested subgroups are presented in **Table 1**. Monolithic zirconia crowns exhibited significantly higher fracture strength than bilayered crowns, regardless of the cement used. The AR (monolithic with resin cement) and AG (monolithic with GIC) groups recorded mean values of **2541 ± 349 N** and **2578 ± 339 N**, respectively. In contrast, the BR (bilayered with resin cement) and BG (bilayered with GIC) groups showed lower strength values of **1557 ± 418 N** and **1691 ± 526 N**, respectively (Table 1).
The **one-way ANOVA** test revealed statistically significant differences among the four groups ($F(3,28) = 13.69$, $\mathbf{p < 0.001}$), confirming that the type of crown had a significant influence on fracture strength, while the cement type did not had any significant effect (Table 2).
Further **post hoc Tukey's multiple comparison** test confirmed significant differences between:

- AR vs BR (mean difference = 984.0 N, **p = 0.0003**),
- AG vs BR (mean difference = 1022.0 N, **p = 0.0002**),
- AR vs BG (mean difference = 849.1 N, **p = 0.0018**), and
- AG vs BG (mean difference = 886.9 N, **p = 0.0011**).

However, there was **no statistically significant difference** between AR and AG (**p = 0.9978**) or between BR and BG (**p = 0.9148**) as shown in **Table 3**.
Scanning electron microscopic (SEM) analysis revealed that all monolithic crowns fractured in a catastrophic manner, exhibiting complete separation across the structure. Conversely, bilayered crowns primarily demonstrated chipping and delamination of the veneering ceramic without core fracture, indicating a different failure pattern (Table 4).
These results indicate that the **monolithic crowns** provide superior fracture strength performance compared to **bilayered crowns**, and the choice between **resin cement and glass ionomer cement** had no significant influence on the outcomes within each crown type (Table 5).

Table 1. Descriptive Statistics of Fracture Strength Values (N = 8 per subgroup)

Subgroup	Crown Type	Cement Type	Mean Fracture Strength (N)	Standard Deviation (±SD)
AR	Monolithic Zirconia	Adhesive Resin Cement	2541	349
AG	Monolithic Zirconia	Glass Ionomer Cement	2578	339
BR	Bilayered Zirconia	Adhesive Resin Cement	1557	418
BG	Bilayered Zirconia	Glass Ionomer Cement	1691	526

Table 2. One-Way ANOVA Summary for Fracture Strength Comparison

Source of Variation	SS	df	MS	F (3, 28)	P value
Between Groups (Treatment)	7,078,423	3	2,359,474	13.69	<0.001***
Within Groups (Residual)	4,824,923	28	172,319		
Total	11,903,346	31			

Table 3. Tukey's Multiple Comparison Post Hoc Test

Comparison	Mean Difference (N)	95% CI of Difference	Significance	Adjusted P Value
AR vs AG	-37.7	-604.4 to 529.0	ns	0.9978
AR vs BR	984.0	417.3 to 1551	***	0.0003
AR vs BG	849.1	282.4 to 1416	**	0.0018
AG vs BR	1022.0	455.0 to 1588	***	0.0002
AG vs BG	886.9	320.2 to 1454	**	0.0011
BR vs BG	-134.8	-701.5 to 431.9	ns	0.9148

Legend:
ns = not significant, ** = significant at $p \leq 0.01$, *** = significant at $p \leq 0.001$

Table 4. Summary of Fracture Mode Observed Under SEM

Group	Crown Type	Observed Failure Mode
A	Monolithic Zirconia	Catastrophic complete fracture
B	Bilayered Zirconia	Chipping and delamination of veneer

Table 5. Summary of Key Findings

Outcome	Observation
Highest fracture strength	Monolithic crowns (AG: 2578 N, AR: 2541 N)
Lowest fracture strength	Bilayered crowns (BR: 1557 N, BG: 1691 N)
Influence of cement type	No significant difference within same crown type (AR vs AG, BR vs BG)
Statistically significant differences	Between monolithic and bilayered crowns regardless of cement type
SEM Failure Mode	Monolithic: complete fracture; Bilayered: cohesive and adhesive veneer loss

DISCUSSION

Because they look good, are safe for the body and are more resistant to wear, zirconia crowns are popular option over traditional metal-ceramic alternatives [1]. We attempted to examine and contrast the fracture resistance of monolithic and bilayered zirconia crowns placed on teeth using adhesive resin cement or regular glass ionomer cement. It was found that monolithic zirconia crowns demonstrated much greater strength compared to bilayered crowns, no matter which cement was used.

The high structural strength of monolithic crowns is partly due to eliminating the weak spot between the core and the veneer that occurs in bilayered crowns [2,3]. The majority of design experts say this interface commonly fails, usually by chipping or delamination, especially when the restoration is being used [4]. Cracks in yttria-stabilized tetragonal zirconia are harder to spread because of the toughness brought by transformation toughening under stress.

As previous literature has found, all types of crowns applied in this study could withstand loads higher than both the average masticatory force and the strongest bite force typically measured [7,8]. This proves that either restorations can be used satisfactorily for strengthening molars.

Even though bilayered zirconia crowns showed less resistance to fracture than some other types, they are valuable when looking for a crown that can be customized in layers. In addition, the findings show that there is a risk of veneer failure, mainly if non-anatomical copings are used since they may not be strong enough to hold the veneer (9). According to research, the success of bilayered systems depends on suitable core shapes, constant veneering thickness and maintained temperatures between the core and veneering materials 10,11.

The current research found no statistically meaningful difference between adhesive resin cement and glass ionomer cement in every crown group considered. Both cements gave similar mechanical support for zirconia crowns, when tested in laboratory conditions. Rosentritt et al. [12] also found that the fracture strength of zirconia monolithic crowns was similar regardless of the cement type used.

However, a number of researchers have preferred adhesive resin cements that include MDP (10-Methacryloyloxydecyl dihydrogen phosphate) as a functional monomer for its better bonding with treated zirconia surfaces, obtained by sandblasting and using a primer. At the same time, conventional cements are easier to manage but depend on small-scale bonding that might not last over time in certain clinical circumstances [15].

Thermocycling was used in this investigation to create the different temperature fluctuations found in the mouth. This type of test has been found to result in lower temperature damage for zirconia which may affect its mechanical characteristics (16). Notwithstanding this, the values for fracture strength exceeded what is required in the clinic, showing zirconia to be reliable for restoration even as samples aged artificially.

Regarding failure modes, groups showed different ways of failing. The majority of monolithic crowns showed complete failure, implying that they broke only once the material was severely overloaded. Conversely, in bilayered crowns, chipping and separation of the porcelain veneer which is weakest in layered crowns, was observed, as documented in other scientific studies.

In general, the results support previous studies that show monolithic zirconia crowns perform better mechanically. They also show that adhesive techniques and conventional cementation methods can be safely and successfully used with zirconia restorations. Even so, you should consider that bilayered crowns have certain mechanical drawbacks because of their attractive appearance.

CONCLUSION

No matter what cement was used, monolithic zirconia crowns were shown to be much stronger against fracture than bilayered zirconia crowns. The fracture resistance of zirconia restorations cemented with adhesive resin and glass ionomer cement was acceptable, so either can be trusted for this treatment. Its strong structure and resistance to failures make monolithic zirconia preferred for posterior crowns.

DECLARATIONS

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Consent for publication

Informed consent was obtained from every participant for documentation and examination.

Competing interests

The authors declare no competing interests.

Ethical approval

Ethical approval was granted by the Institutional Human Ethical Committee

Informed patient consent

All patients' clinical records were obtained with informed consent.

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ORIGINAL RESEARCH

MORPHOFUNCTIONAL FEATURES OF THE ORAL MUCOSA AND ITS REGENERATIVE POTENTIAL IN THE AGE ASPECT

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ABSTRACT

Background: Because they are considered biocompatible, tough and look great, zirconia crowns are commonly used instead of metal-ceramic restorations. The advantage of monolithic zirconia is that it does not include a veneer which can easily chip in bilayered restorations. Cementation technique can affect the toughness of the material. The purpose of this study was to compare between the fracture strength of monolithic and bilayered zirconia crowns cemented with either resin or glass ionomer cements.

Methods and Materials: Sixteen monolithic zirconia crowns and Sixteen bilayered zirconia crowns were fabricated using CAD/CAM technology and formed two groups called Group A and Group B. Each group was split into two new subgroups (eight each) dependent on the type of used cement, naming the subgroups with AR and BR groups for the adhesive resin cement and AG and BG groups for the glass ionomer cement. All crowns were prepared by air-abrasion and cemented onto epoxy resin dies, after which they were thermocycled (5,000 times, from 5°C to 55°C). Strength at fracture was determined on a universal testing machine and the types of fracture failure were explored using a scanning electron microscope.

Results: The fracture strength was much higher in monolithic zirconia crowns than in bilayered crowns, no matter the cement used. The average fracture strength was 2541 ± 349 N for AR, 2578 ± 339 N for AG, 1557 ± 418 N for BR and 1691 ± 526 N for BG. There were no major differences between adhesive and conventional cements used in crowns of the same type ($p > 0.05$). Even so, there were significant differences ($p < 0.001$) between crowns made from solid material and those made from two layers. Analysis of failures showed that monolithic crowns suffered major fractures, but the layer-separation problem arises with bilayered crowns.

Conclusion: The fracture resistance of monolithic zirconia crowns exceeded that of bilayered zirconia crowns, with this advantage noticeable regardless of the cementation method. Both adhesive resin and glass ionomer cements worked well clinically. For posterior restorations that are best suited to strong crowns, monolithic zirconia may be recommended.

Keywords: aging of the oral mucosa, oral aging, regeneration of the mucous membrane, age-related features of the mucous membrane, oral mucosa

INTRODUCTION

Modern dentistry faces a significant challenge—the rapid increase in elderly patients, which requires revising traditional treatment approaches. Recent advances in aging biology have provided new insights into age-related changes in the oral cavity, which are complex and progressive. The oral mucosa in the elderly undergoes physiological transformations: the epithelial layer thins, cellular renewal activity decreases, and protective properties change. Over time, these processes create a favorable environment for various pathologies.

Epidemiological data show a clear correlation between age and the frequency of dental diseases—older patients exhibit more pronounced and diverse oral lesions. Each case requires an individualized approach, considering not only local manifestations but also overall health. Treating elderly patients with multiple chronic conditions is particularly challenging, as it requires balancing treatment efficacy and safety.

Modern clinical guidelines emphasize the importance of preventive measures and gentle treatment techniques aimed at preserving natural teeth and maintaining quality of life. Interdisciplinary collaboration between dentists and other specialists is increasingly important for developing comprehensive care programs for elderly patients. These changes in gerontological dentistry open new opportunities for improving the dental health of the growing elderly population.

The oral mucosa (OM) is a highly specialized tissue consisting of three layers: epithelium, lamina propria, and submucosa [1, 2]. It serves as the first line of defense against external pathogenic influences and performs multiple functions, including barrier, sensory, secretory, immune, and thermoregulatory roles [3–5]. The OM has a high regenerative capacity, essential for recovery from daily mechanical and physiological damage. The epithelium and shedding of its outer layer ensure constant renewal. Newly formed cells maintain mucosal balance, while the removal of surface cells eliminates microorganisms. The protective function is also linked to specialized immune cells [5].

This systematic review presents the morphofunctional features of the OM, highlights the characteristics of its protective barrier, and examines its regenerative capacity in the context of aging. Special attention is given to the mechanisms underlying mucosal regeneration. The review also explores molecular factors in mucosal protection. Two key questions are addressed:

1. Are there structural differences in the OM between young and elderly individuals?
2. Does age affect the regenerative potential of the OM?

The aims of the study: To analyze available data on the structure, function, and regeneration of the OM in the age aspect.

METHODS

The methodology of this study complies with the PRISMA guidelines for systematic reviews and meta-analyses.

Data Sources:

Publications were searched in four electronic databases (PubMed, ScienceDirect, Google Scholar, and eLibrary) from 2014 to 2025.

Search strategy

The following keywords were used in the search (in Russian and in English): the structure of the oral mucosa, oral epithelium, intercellular junctions, epithelial barrier, wound healing, mucosal regeneration, saliva, immunological aspects of regeneration, influence of factors on regeneration, aging of the mucous membrane, aging of the oral mucosa, oral microbiome, regenerative potential of the mucous membrane. In addition, the bibliographic sources of the found publications were studied and suitable studies were additionally manually selected from them.

Data collection

The search was performed by five independent researchers and was last updated on March 25, 2025. Two independent reviewers (DAM & TGT) reviewed the titles and abstracts of each study to determine its compliance with the established inclusion and exclusion criteria. There were no restrictions on the language of publications.

Potentially eligible studies underwent a full-text review, and discrepancies between reviewers were resolved through discussion. First, articles obtained from the database search were identified; then, articles were selected, excluding duplicates and those not relevant to certain descriptors, by screening titles and abstracts; after this step, an eligibility check was performed with full text reading, excluding articles that did not meet the previously established criteria; finally, relevant articles were included in the review.

Inclusion criteria

The publications present the results of in vitro and in vivo studies, including: randomized controlled trials, controlled clinical trials, observational studies, multicenter studies, comparative studies, and literature reviews. The research included the results of studying the structure of the oral mucosa in the age aspect, the aging of the oral mucosa, the processes of wound healing of the mucous membrane in normal and pathological conditions, the influence of various factors on the regenerative abilities of the mucous membrane, changes in the regenerative potential of the oral mucosa with age.

There were no restrictions on minimum quality, minimum sample size, or number of patients.

Exclusion criteria

Publications were excluded from the review if: the age of the subjects was under 18; descriptive works without clearly structured results and conclusions; studies with insufficient information to extract data; letters to the editor; comments; unpublished works.

The effectiveness was assessed by summarizing the relevant data on the results obtained in the course of individual studies.

Data extraction

Six reviewers (DAM, TGT, EGM, MDS, GLS, NDG) extracted data from the included studies using a standardized data extraction form. Reviewers identify and document the threats to the validity of each study due to faulty execution or poor measurement. Discrepancies in data extraction were resolved by discussion.

Quality assessment

The effectiveness was assessed by summarizing the relevant results obtained in the course of individual studies. The aim of the study was to systematize information about the oral mucosa and its regenerative potential in the age aspect.

Data synthesis and analysis

The results of this review were reported following the PRISMA guidelines. A narrative synthesis of the findings was provided. Six independent reviewers (DAM, TGT, EGM, MDS, GLS, NDG) the texts, selecting texts that met the inclusion criteria. The year of publication, study methodology, population (number of patients, mean age of patients), study results, outcome.

Effect measures

The effectiveness was evaluated by synthesizing relevant outcome data extracted from selected studies. The outcome was to systematize information of the features of the structure, functioning and regeneration of the oral mucosa in the age aspect.

Risk of bias

The form collects information needed to monitor the status of screening, reviewing and summarizing each article by 4 reviewers. Developing tables that summarize the body of evidence. The form captures detailed descriptive data about the intervention and evaluation. Classifying other key characteristics of the intervention and assessing the quality of the study's execution. Reviewers identify and document the threats to the validity of each study due to faulty execution or poor measurement. This information is used as a criterion for continued inclusion of the study in the body of evidence for intervention.

Following the study design, it has identified domains rating the certainty of evidence: risk of bias, inconsistency and publication bias. To assess the risk of bias, each individual criterion was considered to have a low risk of bias, a high risk of bias, or an uncertain risk of bias (lack of information or uncertainty related to potential bias). Discrepancies between authors were resolved by consensus.

Clinical outcome

In the literature review, information of the features of the structure, functioning and regeneration of the oral mucosa in the age aspect.

Results of the search

Based on the criteria, 66 publications were selected and included in the systematic review. The research included the results of studying the structure of the oral mucosa in the age aspect, the aging of the oral mucosa, the processes of wound healing of the mucous membrane in normal and pathological conditions, the influence of various factors on the regenerative abilities of the mucous membrane, changes in the regenerative potential of the oral mucosa with age.

RESULTS

Initially, 8,052 publications were screened by date, title, and abstract. Duplicates were removed, leaving 5,874 unique publications. After reviewing titles, abstracts, and conclusions,3,298 publications were excluded for irrelevance. The selection process is illustrated in Figure 1.

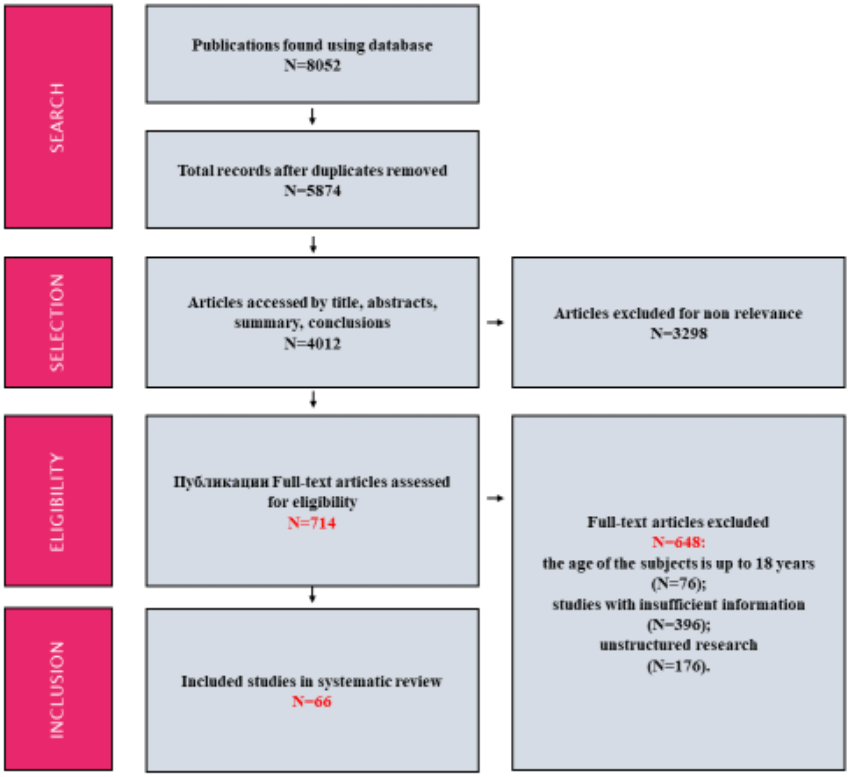


Figure 1. Criteria for selecting publications

Disagreements on inclusion were resolved through discussion. Based on the criteria, 66 publications were selected for the systematic review.

DISCUSSION

The oral mucosa exhibits remarkable heterogeneity, reflecting its adaptation to a variety of functional loads. According to classical works [5,6], histologists distinguish three main types of mucosa: masticatory, lining, and specialized, each of which has unique structural characteristics. These differences are especially noticeable when analyzing multilayer epithelium, represented by three forms: non-keratinized, orthokeratous (complete keratinization) and parakeratous (incomplete keratinization).

Areas subject to intense mechanical stress, such as the hard palate and gums, are covered with chewing mucosa. Orthokeratous epithelium prevails here, firmly connected to the underlying tissues by dense collagen fibers, which

provides resistance to abrasion. In contrast, the lining mucosa located on the cheeks, soft palate, lips, and bottom of the oral cavity is formed by a non-corneal epithelium supported by elastic connective tissue, which allows the tissues to stretch freely with jaw movements. The parakeratous type, characteristic of areas with moderate stress (for example, areas of the cheeks along the line of teeth closure), is an intermediate variant with partial keratinization. Interestingly, about half of the oral cavity area is covered by keratinizing forms of epithelium, while 30% is occupied by non-keratinizing, and the remaining 20% is occupied by teeth [3,7–9].

A special place is occupied by the specialized mucous membrane of the back of the tongue, combining elements of keratinized and non-keratinized epithelium. Its surface forms a complex relief due to the lingual papillae, which perform mechanical and sensory functions, and taste buds are integrated into this structure, providing chemoreception. The rate of epithelial cell renewal varies depending on the location: proliferation occurs more intensively in the lining areas than in the chewing areas. This dynamic is supported by the active cell division of the basal layer, which allows the mucosa to recover quickly despite constant mechanical and microbial influences [3,7–9].

The mucous membrane of the mouth is exposed to frequent mechanical effects of solid food, temperature factors, biting, exposure to microorganisms, etc. As a result, one of the main functions of the oral mucosa is protective. It is provided largely due to the unique architectural integrity of the multilayer epithelium, where epithelial cells are connected by tight junctions (TJs) (occlusal junctions), gap junctions and anchor junctions (desmosomes and adhesive junctions); and also due to the constant renewal of the multilayer epithelium, its excellent regenerative ability [10,11,12,13]. The production of cells in the deeper layers of the epithelium is balanced by the loss of cells from the surface. There is a rapid purification of surface cells, which acts as a protective mechanism, limiting the colonization and invasion of microbes adhering to the surface of the mucous membrane [8].

The problem of regeneration of the oral mucosa is currently an important problem of modern dentistry and carries a number of important issues, how does the healing process occur normally and in pathological conditions of OM, what factors influence it; what changes occur in OM with age, as well as the features of its regenerative potential in the age aspect.

Mucosal repair includes hemostasis, inflammation, proliferation, and remodeling [14].

Immediately upon damage to the OM and rupture of blood vessels, the coagulation cascade is activated to prevent blood loss and ensure temporary sealing of the wound. The opened extracellular matrix causes the activation of circulating platelets, which further triggers the hemostatic cascade. Platelets, surviving keratinocytes, and fibroblasts produce biologically active substances such as vasoactive mediators and chemotactic signals that release proteases, cytokines, and growth factors to quickly trigger the inflammatory phase, attracting immune cells to the area of damage. Blood vessels constrict to prevent bleeding, and platelets clump together to form platelet plugs, which strengthen by polymerizing fibrin, forming a fibrin clot and closing the wound. Fibro-fibronectin clots provide support as a temporary matrix for the extracellular matrix and allow epithelial cells and fibroblasts to migrate to the wound area [15,16].

The inflammation phase occurs almost immediately after blood clotting and lasts 2-4 days [17]. It is aimed at removing dead tissues from the damaged area and preventing infection by pathogenic microorganisms. Damage-associated molecular patterns (DAMP) and pathogen-associated molecular patterns (PAMP) in the wound bed trigger Toll-like receptor (TLR), multiligand receptor (RAGE), and inflammasome signaling pathways, leading to a cascade of cytokine and chemokine release by resident cells, which marks the beginning of the inflammatory phase in within a few hours of injury. Neutrophils, monocytes, macrophages, mast cells, and T-cells penetrate the wound bed in response to chemokine gradients and trigger an immune response against potential pathogens [18]. As early as 4 hours after injury, keratinocytes, fibroblasts, Langerhans cells and resident macrophages begin to phagocytize the formed dead cells. Primary keratinocytes and fibroblasts of the oral cavity produce more interleukin 8 (IL-8, also known as CXCL8) after stimulation by early inflammatory mediators such as tumor necrosis factor alpha, interferon gamma, interleukin-1 beta (TNF-α,

IFN-γ, IL-1β), in addition, keratinocytes increase expression ICAM-1 (adhesion molecules used by neutrophils to facilitate extravasation and migration to the damaged area), which promotes a faster influx of immune cells into the damaged area and thus accelerates wound healing [19,20].

The proliferation phase includes several processes, such as angiogenesis, proliferation and differentiation of fibroblasts into myofibroblasts (formation of granulation tissue) and proliferation of epithelial cells (re-epithelialization) [17]. During the proliferation phase, which lasts from several hours to several days after injury, endothelial cells, fibroblasts, and epithelial cells migrate to the wound surface for tissue regeneration. Well-vascularized and loose granulation tissue formed by fibroblasts and endothelial cells provides the wound surface with structural support and nutrition. Meanwhile,

epithelial cells repair the wound surface epithelium during proliferation, migration, and differentiation [21]. Stagnation of blood in the damaged area leads to hypoxia and triggers an angiogenic reaction. Cells in the hypoxic wound bed secrete various pro-angiogenic factors, such as hypoxia-induced factor 1a (HIF-1a) and vascular endothelial growth factor (VEGF), which stimulate the proliferation and migration of endothelial cells, resulting in the formation of a large number of permeable vessels that provide granulation tissue with nutrients and immune cells. During wound healing, angiogenesis is accompanied by the creation of a dense but poorly organized capillary bed, which eventually shrinks to a normal density and capillary architecture [22].

In wounds of the oral cavity, there is a lower level of infiltration of macrophages, neutrophils and T cells with less secretion of inflammatory cytokines, including interleukin-6 (IL-6) and other factors produced by keratinocytes. These data suggest that attenuation of the inflammatory response is a key feature of wound healing. The epithelium of the mouth has a lower expression of transforming growth factor beta-1 (TGF-β1), a proinflammatory and profibrotic cytokine, which is known to promote the formation of hypertrophic scars during wound healing [23]. A smaller number of immune cells in a healthy oral mucosa can cause a weakened immune response upon injury, suggesting that the mucosa is inherently less sensitive to inflammation, and may explain its higher healing ability [24,25,26].

Keratinocyte migration and proliferation are key processes during re-epithelialization, as they are less prone to differentiation and instead preferentially adopt a proliferative and migratory phenotype.

Oral keratinocytes exhibit accelerated migration (2.6 times faster than skin keratinocytes) and higher proliferation, independent of the influence of

underlying connective tissue, which is their innate feature [27].

In addition, oral fibroblasts produce more hepatocyte growth factor (HGF) and keratinocyte growth factor (KGF) (both are known inducers of keratinocyte migration and proliferation) [28,29].

The oral mucosa is constantly washed with saliva, which contains epidermal growth factor (EGF), which accelerates re-epithelialization, and fibroblast growth factor (FGF), which acts on fibroblasts, increasing their renewal and promoting wound healing. Saliva also contains histatins, hydrogen peroxide, lactoferrin and lysozymes, antimicrobial peptides and mucins, which provide essential antimicrobial protection and can promote wound healing by stimulating fibroblast proliferation and migration, accelerating keratinocyte renewal and releasing growth factors [23,30,31,32,33].

Histatins, in particular histatin-1, promote cell adhesion and migration in oral keratinocytes, gingival fibroblasts, epithelial cells outside the oral cavity, and endothelial cells. This is especially important because histatin-1 promotes the phase of re-epithelialization and angiogenic reactions, enhancing the migration of epithelial and endothelial cells [34,25,35,36].

Nerve growth factor (NGF) and its various precursor forms are secreted by the salivary glands into human saliva, and are also produced by a multitude of cells in the tissues of the oral cavity. The main forms of NGF in human saliva are forms of pro-nerve growth factor (pro-NGF), rather than mature NGF. NGF receptors associated with tropomyosin kinase A (TrkA) and neurotrophin p75 receptor (p75NTR) are widely expressed in human oral soft tissue cells, including keratinocytes, endothelial cells, fibroblasts and leukocytes, as well as in ductal and acinous cells of all types of salivary glands. NGF is involved in most stages of the oral wound healing process: repair, cell survival, apoptosis, cell proliferation, inflammation, angiogenesis, and tissue remodeling [37].

While keratinocytes migrate and multiply, restoring the epithelial barrier, granulation tissue is formed. Fibroblasts and endothelial cells begin to secrete fibronectin to create a dense fibrillar network that ensures cell migration to the wound surface. It was found that more fibronectin, fibronectin ED-A, and chondroitin sulfate are expressed in OM, and less elastin is expressed [38]. A dense network of fibronectin at the wound site is necessary for the formation of a mature collagen matrix I/III [39]. In the initial stages of wound healing, the main type of collagen produced is collagen III, followed by the production of collagen I to increase the strength and elasticity of tissues [39,40].

In addition to the fibrous proteins that form the structural basis for cells, the extracellular matrix contains many matrix proteins (such as glycoproteins and proteoglycans) that promote the matrix's interaction with cells and regulate various cellular reactions such as adhesion, migration, and cell proliferation [40].

As soon as the wound is covered with new epithelium and the balance between the body and microbes is restored, the inflammation is suppressed by anti-inflammatory cytokines. Macrophages switch from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype and secrete growth factors, matrix metalloproteinases (increased expression of MMP1, MMP2, MMP3, and MMP10), and tissue metalloproteinase inhibitors (TIMPs), stimulating fibroblast-mediated remodeling of the extracellular matrix [18]

Human OM fibroblasts have higher proliferation and weaker contractility, and also exhibit delayed aging due to their long telomere length. An embryonic or fetal phenotype associated with a long telomere may be the

reason that wounds on the mucous membrane heal with less scarring [23]. Regarding the properties of cell adhesion, fibroblasts of the oral mucosa demonstrate higher adhesion to vitronectin and collagen types I and IV, and are very sensitive to transforming growth factor beta (TGFb1), the main profibrotic cytokine expressed during healing. TGFß1 increases the rate of proliferation, as well as collagen synthesis [38].

There is increasing evidence that the oral mucosa may contain a population of progenitor cells or "stem-like" cells, known by various names, including progenitor cells of the mucosal lamina proper, stem cells, and mesenchymal gum stem cells, which may contribute to reducing scarring observed during wound healing [41]. The ability of the oral mucosa to recover quickly without scarring is determined not by any one feature, but by key external and internal factors present at all stages of the wound healing process, which are crucial for improving the final result [42,43,44]. Human stem cells obtained from the oral cavity have a plasticity and ability to proliferate 30-50% higher, as well as a more pronounced immunoregulatory effect compared to bone marrow stem cells. Due to their potential for differentiation, mesenchymal stem cells derived from oral tissues are promising for tissue engineering and regenerative medicine [45,46,47].

Tissue remodeling (the contraction phase, «maturation») is the last and longest phase of tissue healing; it consists of collagen synthesis and degradation to align newly formed collagen bundles along tension lines and can last from several weeks to several months [17].

Adaptive mechanisms that ensure not only effective restoration of tissue integrity, but also the prevention of chronic processes against the background of constant microbial colonization are described in detail in studies [48,49]. The integration of these systems makes it possible to maintain the functionality of the dental apparatus even under regular mechanical stress, demonstrating the amazing resistance of the complex ecosystem of the oral cavity. Depletion of reserves, reduction of biodiversity, and gradual specialization within the oral ecosystem eventually lead to a significant decrease in its ability to cope with everyday tasks [50].

On the one hand, some studies show that there is no evidence to support that age is a factor in poor wound healing. However, there is evidence that wound healing is slower in the elderly than in the young; the number of fibroblasts decreases with age, which is associated with their proliferative activity [41]. In addition, elderly people exhibit an altered inflammatory response characterized by a steady increase in the level of pro-inflammatory cytokines such as IL-6 and TNFa, and a decrease in the level of growth factors. This combination leads to a high level of TGF-β, which may play a role in the transformation of acute wounds into chronic ones by suppressing re-epithelialization [35]. Accelerated and progressive aging is also associated with delayed infiltration of macrophages and T cells into the wound area and decreased macrophage function [26,27]. Microcirculation disorders and hypoperfusion, characteristic of aging tissue, contribute to the weakening of the inflammatory response and interfere with the physiological angiogenic phase as a whole, and reduced extracellular matrix production and increased expression of matrix metalloproteinases, especially MMP-2, leads to disorders in the remodeling phase [51].

From a clinical point of view, the oral mucosa in the elderly may not differ from the oral mucosa in young people. However, its appearance may change due to various factors affecting the oral cavity over a long period of time. The balance of the various ecological niches of the oral cavity is the result of a large number of interrelated factors [50]. Local factors include mechanical stimuli, salivation insufficiency, and ear diseases. Systemic risk factors include metabolic disorders such as chronic nutrient deficiencies, including vitamins and trace elements, as well as endocrine dysfunctions, in particular diabetes mellitus. No less significant are cardiovascular disorders, for example, arterial hypertension and ischemic conditions, which provoke a deterioration in peripheral blood circulation, which directly affects tissue trophism. Pathologies of the gastrointestinal tract, accompanied by impaired nutrient absorption and dysbiosis, also contribute to the destabilization of oral homeostasis. Prolonged use of pharmacological agents with a xerostomicor ulcerogenic effect potentiates structural changes in the epithelium. The use of tobacco and alcohol not only disrupts microcirculation, but also suppresses local immunity, creating prerequisites for chronic inflammation. Improper oral hygiene, expressed in irregular or inadequate procedures, contributes to the accumulation of bacterial plaque. The synergy of these factors leads to a cumulative effect: for example, the combination of vitamin deficiency and smoking accelerates mucosal atrophy, and hyperglycemia while taking certain medications exacerbates xerostomia [52-55]. Sex hormones play a role in the physiological healing of wounds. In addition, there are sexual features of wound healing in the elderly: the healing of acute wounds in older men is much slower than in older women due to the positive regulatory effect of estrogen on wound healing [51].

During the aging process, progressive atrophic processes develop in the integumentary epithelium of the OM. The thickness of the epithelial layer is significantly reduced: in the lip area — from 500 to 300 microns, cheeks — from 700 to 400 microns, on the back of the tongue — from 800 to 500 microns. The

cells of the basal layer undergo structural metamorphoses — their shape becomes low-prismatic, the nuclei decrease in size, acquiring a pyknotic appearance, which is accompanied by a decrease in the content of DNA, RNA and dehydrogenase activity. These changes reflect the suppression of proliferative and metabolic functions, and the smoothing of epithelial scallops disrupts the histological architecture of tissues [56,57]. Visually, OM loses its pink hue, becoming grayish-white due to increased keratinization of the multilayer squamous epithelium. In parallel, there is a decrease in the number of cellular elements and fibrous structures in the underlying connective tissue. Elastic fibers undergo sclerosing and thickening, while collagen fibers undergo hyalinization, losing their order and increasing in diameter. This leads to a decrease in the elasticity and mobility of the mucosa, making it rigid and vulnerable to mechanical influences. The vascular system of the OM also undergoes age-related degeneration: the walls of the vessels become sclerosed, the capillary loops of the gums become less pronounced, and part of the anastomoses is obliterated. The formation of vascular-free zones, recorded during histological analysis[58], exacerbates trophic disorders. Against the background of general tissue dehydration, the mucosa becomes thinner and loses moisture, which is manifested by increased sensitivity and susceptibility to mechanical influences, as well as delayed repair. Even minor injuries, such as denture friction, in elderly patients with concomitant chronic diseases often provoke the formation of painful decubital ulcers, characterized by prolonged healing. Age-related changes create prerequisites for the development of specific pathologies: cracks in the corners of the mouth, candidal lesions, allergic reactions, lichen planus and lichenoid changes. These conditions, which occur in the elderly 3-4 times more often than in the young [59,] are associated with the combined effects of reduced local immunity, impaired microcirculation, and chronic tissue hypoxia.

In patients with complete adentia, age-related transformations of the oral mucosa become more pronounced. Each anatomical region exhibits unique patterns of degeneration reflecting local features of tissue organization. Progressive thinning of the epithelial layer is observed in the buccal region, mainly due to the reduction of the thorny and basal layers. The cells decrease in size, their nuclei become hyperchromic, and the epithelial scallops lose their relief, which disrupts the structural integrity of the mucosa. Molecular studies have revealed a critical decrease in the expression of the SIRT3 and SIRT6 genes in the buccal epithelium. These genes encode sirtuins, proteins that play a key role in protecting cells from oxidative stress and slow down the development of age—related pathologies [60]. Their deficiency exacerbates the processes of cellular aging, creating a vicious circle of degenerative changes. In the gum mucosa, despite the relative preservation of the epithelial layer, other pathological processes are noted. Fibrous changes are accompanied by fragmentation of nerve endings, the density of which decreases by 40-50% with age. Unlike the buccal region, the mucous membrane of the hard palate shows a paradoxical thickening with a simultaneous loss of density, which is associated with the restructuring of the connective tissue stroma. Sclerotic processes prevail here: collagen fibers thicken and are replaced by fatty inclusions, and the number of vessels and cellular elements is reduced by 30-35%. The histological picture is complemented by the appearance of extensive vascular-free zones and areas with a complete absence of cellular elements. These changes are aggravated by trophic disorders, which is especially noticeable in patients with prolonged adentia.

The sensitivity of the oral cavity, defined as the distinction of touch points, does not change significantly with age. Only a slight decrease in sensitivity was observed in people over 80 years of age. Different areas of the oral mucosa exhibit varying degrees of permeability. Increased keratinization is associated with decreased permeability (e.g., hard palate), and decreased keratinization is associated with increased permeability (e.g., the floor of the oral cavity). An in vitro study was conducted showing that the permeability of the cell membranes of the epithelial cells of the oral cavity increases with age [56.] It has been found that tactile sensations in the mouth (i.e., sensitivity and spatial acuity) and perception of warm but not cold stimuli worsen with age, especially after 80 years [19].

The oral microbiota influences wound healing by secreting lipopolysaccharides that support the homeostasis of oral mesenchymal stem cells through the microRNA-21/Sp1/telomerase reverse transcriptase pathway (Su et al., 2018). Bacteria can accelerate wound healing, having a positive effect on the immune response, granulation tissue, and collagen formation [44,61]. It has been proven that aging is one of the factors affecting the structure of the oral microbiota and its sensitivity to various viral, fungal, or bacterial infections [62,63]. Older people may have a higher bacterial diversity than younger and middle-aged people [52]. For example, Liu et al. (2020) examined the microbial composition in three areas of the oral cavity (gum fluid, back of tongue, and saliva) and concluded that the alpha diversity of bacteria decreases with age, while beta diversity tends to increase [64]. Willis et al. (2022), on the other hand, comparing saliva samples in different age groups, they concluded that in middle age the composition of the oral

microbiota is most homogeneous, while in old age there is increased microbial diversity due to low-abundance taxa [65]. The oral microbiome depends on the niche and varies depending on the age group, and also provides new insight into the possible relationship between changes in the oral microbiome and age-related diseases. Studying the age-related features of microbial communities in various parts of the oral cavity can open up new opportunities for microbiome-based diagnosis, new directions in preventive medicine, and offer innovative treatment methods [66].

CONCLUSION

In the context of global demographic aging, an in-depth study of age-related transformations of the oral mucosa is of particular scientific and practical importance. The tissue structures of the oral cavity, being a kind of reflection of systemic age-related changes, undergo a complex transformation requiring detailed morphofunctional analysis.

Modern research reveals a paradoxical feature: despite its apparent accessibility for visual observation, the oral mucosa remains one of the least studied organs in the gerontological aspect. Meanwhile, her age-related changes are systemic in nature:

- progressive atrophy of the epithelial layer;
- reduction of the vascular network;
- reduction of regenerative potential;
- changes in the qualitative composition of saliva and microbiota.

The clinical significance of these transformations cannot be overestimated. Firstly, they create the pathogenetic basis for the development of a number of diseases and conditions (atrophic glossitis, xerostomia, leukoplakia, candidal lesions, lichen planus and others). Secondly, they modify the pharmacodynamics of local medicines, requiring correction of therapeutic approaches. Thirdly, they affect the processes of oral sensory perception, significantly changing the quality of life of elderly patients.

Of particular relevance is the study of the molecular mechanisms of aging of the oral epithelium, such as activation of senescent phenotypes, disruption of intercellular communications, changes in the profile of cytokine secretion, dysregulation of apoptosis.

A promising direction is the development of «age-adapted» diagnostic algorithms and therapeutic protocols that take into account the specifics of the metabolism of the aging oral mucosa. This is especially important in light of recent data demonstrating a link between the condition of the oral epithelium and the risk of developing age-related systemic diseases.

Thus, an in-depth study of the gerontological aspects of the morphofunctional state of the oral mucosa is a strategically important area located at the junction of clinical dentistry, gerontology and fundamental medicine. Further research in this area will not only improve the quality of dental care for elderly patients, but also, possibly, reveal new aspects of the general biological mechanisms of aging.

Authors' Contributions:

D.A. Moiseev (DAM) – research concept/design, data collection/analysis, writing, editing, final approval;
T.G. Tsagaraeva (TGT) – data collection/analysis, writing;
I.S. Kopetsky (ISK) – research concept/design, editing, final approval;
E. G. Mikhailova (EGM) – data collection/analysis;
M. D. Stavitsky (MDS) – data collection;
G. L. Stepanyan (GLS) – data collection;
O.Yu. Guseva (OYG) – analysis;
Yu.L. Vasil'ev (YLV) – supervision, editing;
N.D. Gontar (NDG) – data collection.

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REVIEW ARTICALE

PHYTOCHEMICAL-INFUSED SCAFFOLDS: THE ROLE OF CISSUS QUADRANGULARIS IN ADVANCING BONE TISSUE ENGINEERING: A SYSTEMATIC REVIEW

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ABSTRACT

Introduction: Cissus Quandrangularis has been used in traditional medicine for centuries for its repair of bone fractures, joint health, osteoporosis prevention, antibacterial, analgesic, anti-inflammatory, antioxidant properties. When this herb is mixed in modern day research of bone engineering, the greater demand of bone grafts and graft failure can be met. Therefore this study aimed to assess the effect of Cissus quandrangularis infused scaffolds in bone regeneration in various cell cultures.

Materials and Methods: Last 10 year published english articles from pubmed, scopus, web of science etc were searched for inclusion in the review. Original invitro research investigating the effect of Cissus Quandrangularis based scaffolds on Cell cultures were included. Data extraction was done by two authors and risk of bias was determined by QUIN guidelines

Results: After the data was extracted, 12 studies out of the 51 that were assessed were included. Ten of the twelve research that were included used human cell cultures, while the other two used murine cell lines. Alkaline phosphatase activity was the primary test employed in the included trials for bone regeneration. Nine of the twelve studies that were included employed this.Alkaline phosphatase activity (ALP activity) is a biochemical assay that can be used to detect the enzyme alkaline phosphatase, which is involved in dephosphorylation reactions.

Conclusion: The bone regenerative potential of Cissus quadrangularis-infused scaffolds using both animal and human cell cultures is proved by all the 12 studies with positive results. This avoids the biological complications of allografts and autografts.

Keywords: Cissus quandrangularis; Bone regeneration; Scaffold; Osteoblast activity, Murine Cell line

INTRODUCTION

Bone tissue engineering is an exciting and evolving field that tackles the complex challenges of bone loss and repair. Bone tissue engineering (BTE) seeks to restore bone that has been lost due to trauma, illness, infection, tumors, or genetic conditions by employing advanced treatment and augmentation methods. These interventions are designed to address disruptions in normal biomechanics caused by such losses[1]. The greater demand for bone-grafts with intrinsic osteoinductivity is still a significant confrontation for clinicians.This area blends biology, materials science, and engineering to create innovative solutions for regenerating bone tissue. At the heart of this field are scaffolds—three-dimensional frameworks designed to mimic the natural extracellular matrix and support the growth of new bone tissue [2].

When it comes to bone grafting treatments, bone replacement scaffolds are in great demand as substitutes for autografts and allografts. It makes sense that choosing the right materials for the scaffold is essential since it will support the defect location and promote osteogenesis, or the growth of new bone [3]. Calcium phosphates (CaPs) are considered highly promising for bone replacement due to their chemical similarity to the inorganic component of bone. Among them, β-tricalcium phosphate (βTCP, Ca3(PO4)2) has gained significant attention for its biocompatibility, bioactivity, and biodegradability. Additionally, 3D-printed scaffolds with custom-designed porosity have been employed to more accurately replicate the complex hierarchical structure of natural bone, particularly in the cancellous (spongy) regions. [4].

Recently, integrating phytochemicals into these scaffolds has shown promise for improving their effectiveness. Among these natural substances, Cissus quadrangularis, a plant with a rich history in traditional medicine, has emerged as a particularly interesting candidate for advancing bone tissue engineering [5].

Cissus quadrangularis (CQ), a member of the Vitaceae family, also commonly known as veldt grape and devil's backbone. It has been used in traditional medicine for centuries to treat fractures and promote bone health.Other names for Cissus quadrangularis (CQ) include devil's backbone and veldt grape [6]. With quadrangular-sectioned branches that have internodes that are 8–10 cm (3–4 inch) long and 1.2–1.5 cm (0.5–0.6 inch) wide, the plant can grow up to a height of 1.5 m (4.9 ft). These species are indigenous to Arabia and tropical Asia. In conventional medicine, it is used for the repair of bone fractures, joint health, osteoporosis prevention, antibacterial, analgesic, anti-inflammatory, antioxidant, and tissue protection [7].

Steroids and vitamins included in CQ have been shown to have a particular impact on the repair of bone fractures. Studies on fracture healing indicate that its unknown anabolic steroids might interact with the bone's estrogenic receptors [8]. The photochemical analysis of CQ reveals that it contains high levels of ascorbic acid, carotene, anabolic steroidal compounds, vitamin C, potassium, calcium, zinc, sodium, iron, lead, cadmium, copper, calcium oxalate, and magnesium. Notably, the stem extract of CQ comprises approximately 4% calcium and phosphorus ions by weight, which are essential for bone fracture healing [9,10].

Although the exact active ingredients of CQ extract are still unknown, it is believed that many CQ compounds exhibit synergistic effects. Since β-sitosterol and stigmasterol are precursors of anabolic steroidal compounds, they can bind to osteoblasts and encourage their development and proliferation, making their quantification crucial for treating bone fractures [11,12]. In one study, the plant's phytoestrogens may have contributed to the rise in IGF expression by regulating the effects of CQ on components of the insulinlike growth factor (IGF) system in osteoblasts, which are crucial for bone development and remodeling [13]. Therefore this review aimed to assess the effect of Cissus quandrangularis infused scaffolds in bone regeneration in various cell cultures.

MATERIAL AND METHODS

Protocol

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis 2020 Statement and the Cochrane Handbook were referenced for developing the review's protocol [14,15].

Eligibility Criteria

A PICOS criterion was created [19] to assist in developing the search strategy and choosing the inclusion criteria for the papers in the current systematic review [16].The following is the PICOS formula.

- Population (P) - Different cell culture
- Intervention (I) - Cissus Quandrangularis infused scaffolds
- Comparison (C) - Conventional or other herb based scaffolds
- Outcome (O) - Bone regeneration
- Study Design (S): Original invitro research investigating the effect of Cissus Quandrangularis based scaffolds on Cell cultures were included; Brief communications, summaries, narrative reviews, editor's letters, case reports, and case series weren't.

Only articles that were published were reviewed for this evaluation. An article's publication status or the English
translation that accompanied it determined whether or not it was reviewed.

Information Sources and Search Strategy

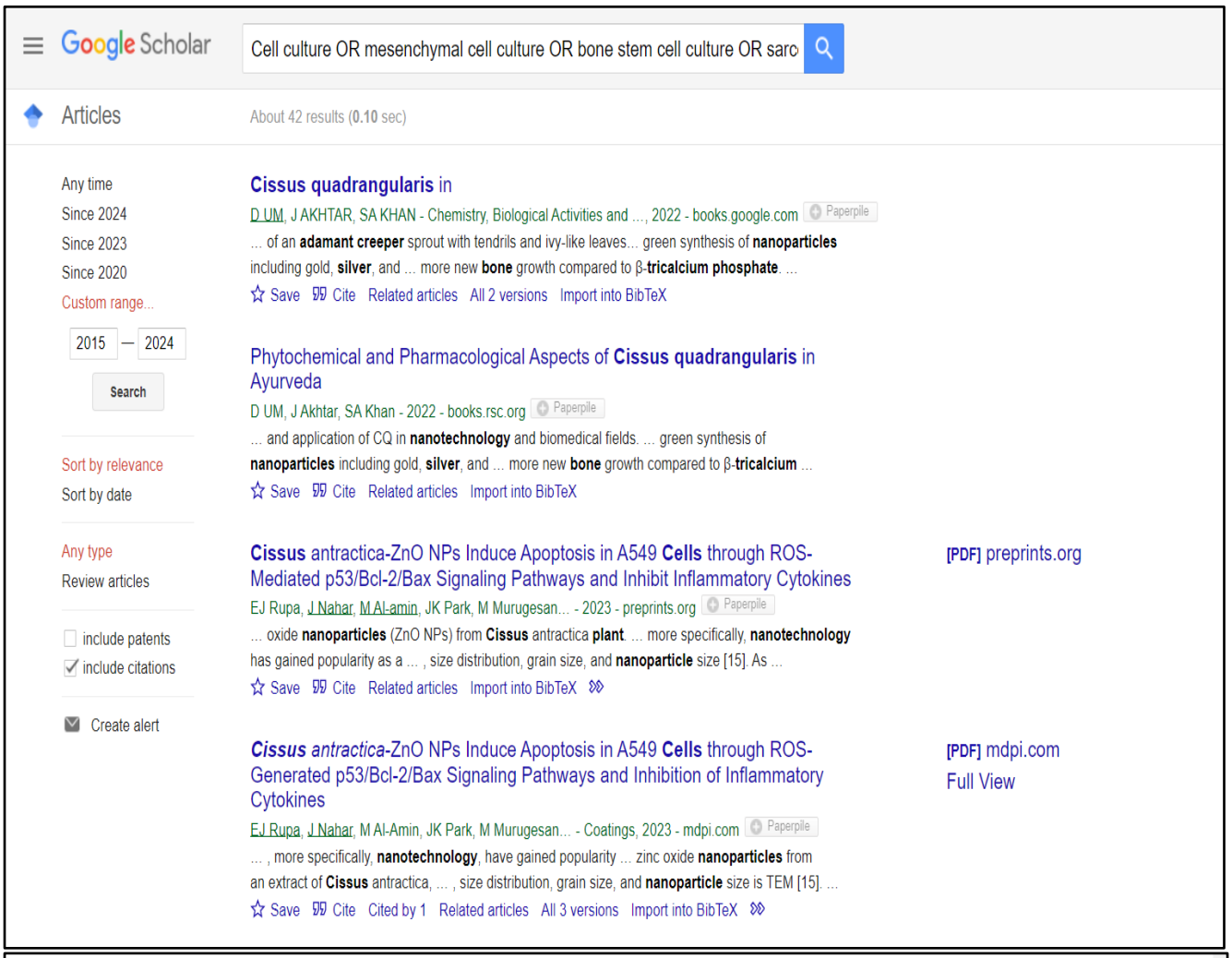
To find all peer-reviewed papers relevant to the issue of the review, a comprehensive search of PubMed, Google Scholar, Cochrane CENTRAL, Scopus, Web of Science, and LILACS was conducted for publications published between January 2009 and June 2024 (15 years). Complex search algorithms were developed taking into account the different vocabulary and grammatical restrictions found in each database.

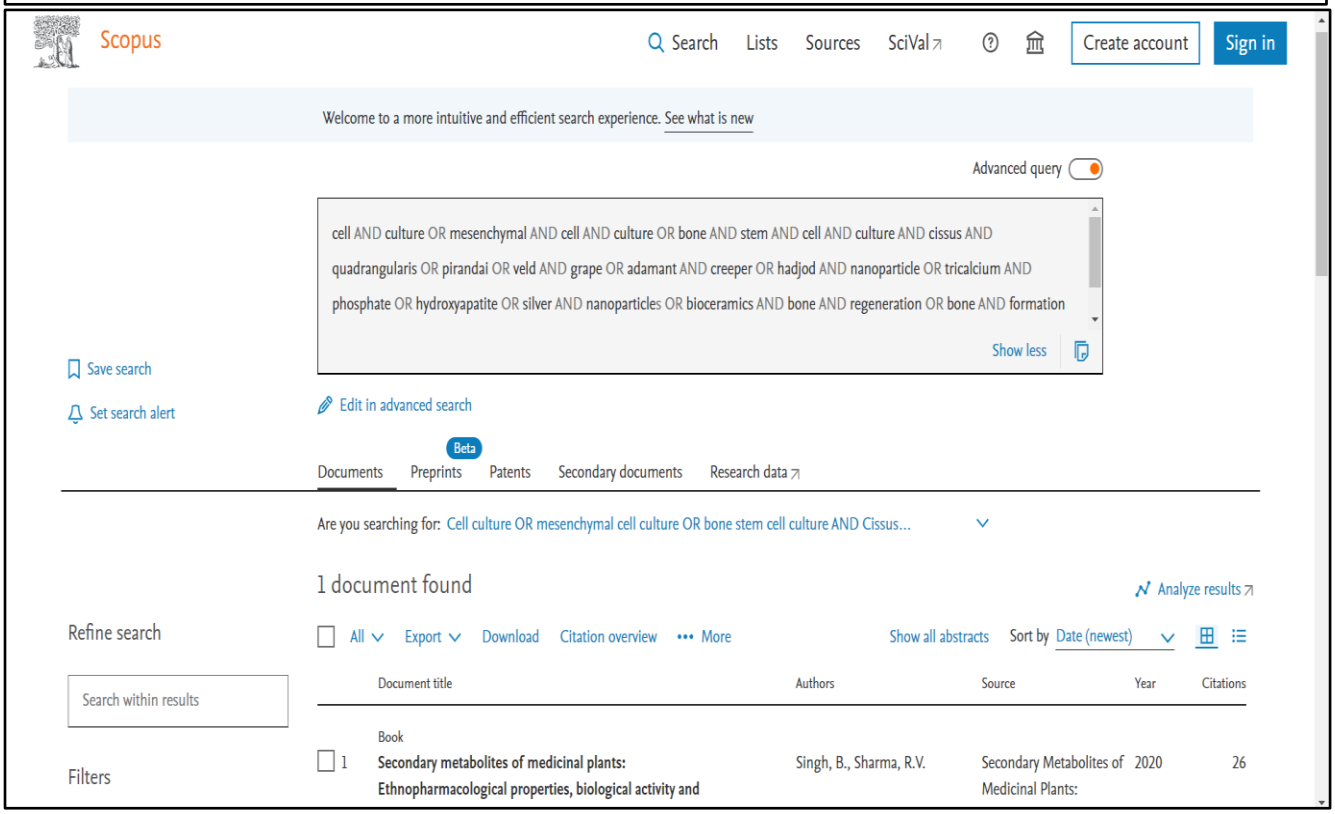
The following MeSH terms were used in search strategy. Cell culture OR mesenchymal cell culture OR bone stem cell culture OR sarcoma cell line OR mesenchymal stem cell OR periodontal ligament stem cell OR bone marrow mesenchymal stem cell OR murine preosteoblast cell line OR umblical cord derived mesenchymal stem cell OR human osteosarcoma cell line OR human bone marrow mesenchymal stem cell OR human osteoblast cell line OR bony defect OR bony defects OR preosteoblast cell line OR human fetal osteoblastic cell line AND Cissus Quadrangularis OR cissus genus OR cissus herb OR pirandai OR Veld Grape OR Adamant Creeper OR Hadjod AND Nanoparticle OR tricalcium phosphate OR hydroxyapatite OR Silver nanoparticles OR graphene nanoparticles OR bioceramics AND Bone regeneration OR bone fORmation OR alkaline phosphatase activity OR osteoblast activity OR osteoblast OR osteoinductive activity OR osteoinductivity OR bone healing OR periodontal regeneration OR intrabony defect regeneration OR osteoclastogenic marker OR osteogenic marker OR regenerative bone tissue engineering OR bone tissue engineering OR osteopontin expression OR Osteogenic potential OR biomineralization. A manual search of several journals, including the Journal of Periodontics, Journal of Clinical Periodontology, Journal of Osseointegration, Journal of Indian Soceity of Periodontology, Clinical Advances in Periodontics and Journal of Periodontolgy and Implant Dentistry was also conducted (Figure 1a-d).

History and Search Details

Download Delete

Search	Actions	Details	Query	Results	Time
#5	•••	>	Search: **#1 AND #2 AND #3 AND #4**	2	00:34:36
#4	•••	>	Search: **Bone regeneration[Title/Abstract] OR bone fORmation[Title/Abstract] OR alkaline phosphatase activity[Title/Abstract] OR osteoblast activity[Title/Abstract] OR osteoblast[Title/Abstract] OR osteoinductive activity[Title/Abstract] OR osteoinductivity[Title/Abstract] OR bone healing[Title/Abstract] OR periodontal regeneration[Title/Abstract] OR intrabony defect regeneration[Title/Abstract] OR osteoclastogenic marker[Title/Abstract] OR osteogenic marker[Title/Abstract] OR regenerative bone tissue engineering[Title/Abstract] OR bone tissue engineering[Title/Abstract] OR osteopontin expression[Title/Abstract] OR Osteogenic potential[Title/Abstract] OR biomineralization[Title/Abstract]**	112,634	00:34:14
#3	•••	>	Search: **Nanoparticle[Title/Abstract] OR tricalcium phosphate[Title/Abstract] OR hydroxyapaptite[Title/Abstract] OR Silver nanoparticles[Title/Abstract] OR graphene nanoparticles[Title/Abstract] OR bioceramics[Title/Abstract]**	113,314	00:33:50
#2	•••	>	Search: **Cissus Quadrangularis[Title/Abstract] OR cissus genus[Title/Abstract] OR cissus herb[Title/Abstract] OR pirandai[Title/Abstract] OR Veld Grape[Title/Abstract] OR Adamant Creeper[Title/Abstract] OR Hadjod[Title/Abstract]**	208	00:19:04
#1	•••	>	Search: **Cell culture[Title/Abstract] OR mesenchymal cell culture[Title/Abstract] OR bone stem cell culture[Title/Abstract] OR sarcoma cell line[Title/Abstract] OR mesenchymal stem cell[Title/Abstract] OR periodontal ligament stem cell[Title/Abstract] OR bone marrow mesenchymal stem cell[Title/Abstract] OR murine preosteoblast cell line[Title/Abstract] OR umblical cORd derived mesenchymal stem cell[Title/Abstract] OR human osteosarcoma cell line[Title/Abstract] OR human bone marrow mesenchymal stem cell[Title/Abstract] OR human osteoblast cell line[Title/Abstract] OR bony defect[Title/Abstract] OR bony defects[Title/Abstract] OR preosteoblast cell line[Title/Abstract] OR human fetal osteoblastic cell line[Title/Abstract]**	116,057	00:17:37


Google Scholar
Cell culture OR mesenchymal cell culture OR bone stem cell culture OR sarc
Articles
About 42 results (0.10 sec)
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Cissus quadrangularis in
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Save Cite Related articles All 2 versions Import into BibTeX
Phytochemical and Pharmacological Aspects of Cissus quadrangularis in Ayurveda
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Cissus antractica-ZnO NPs Induce Apoptosis in A549 Cells through ROS-Mediated p53/Bcl-2/Bax Signaling Pathways and Inhibit Inflammatory Cytokines
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EJ Rupa, J Nahar, M Al-amin, JK Park, M Murugesan... - 2023 - preprints.org
... oxide nanoparticles (ZnO NPs) from Cissus antractica plant ... more specifically, nanotechnology has gained popularity as a ... , size distribution, grain size, and nanoparticle size [15]. As ...
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Cissus antractica-ZnO NPs Induce Apoptosis in A549 Cells through ROS-Generated p53/Bcl-2/Bax Signaling Pathways and Inhibition of Inflammatory Cytokines
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... , more specifically, nanotechnology, have gained popularity ... zinc oxide nanoparticles from an extract of Cissus antractica, ... , size distribution, grain size, and nanoparticle size is TEM [15]. ...
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Advanced query
cell AND culture OR mesenchymal AND cell AND culture OR bone AND stem AND cell AND culture AND cissus AND quadrangularis OR pirandai OR veld AND grape OR adamant AND creeper OR hadjod AND nanoparticle OR tricalcium AND phosphate OR hydroxyapatite OR silver AND nanoparticles OR bioceramics AND bone AND regeneration OR bone AND formation
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Singh, B., Sharma, R.V.
Secondary Metabolites of Medicinal Plants:
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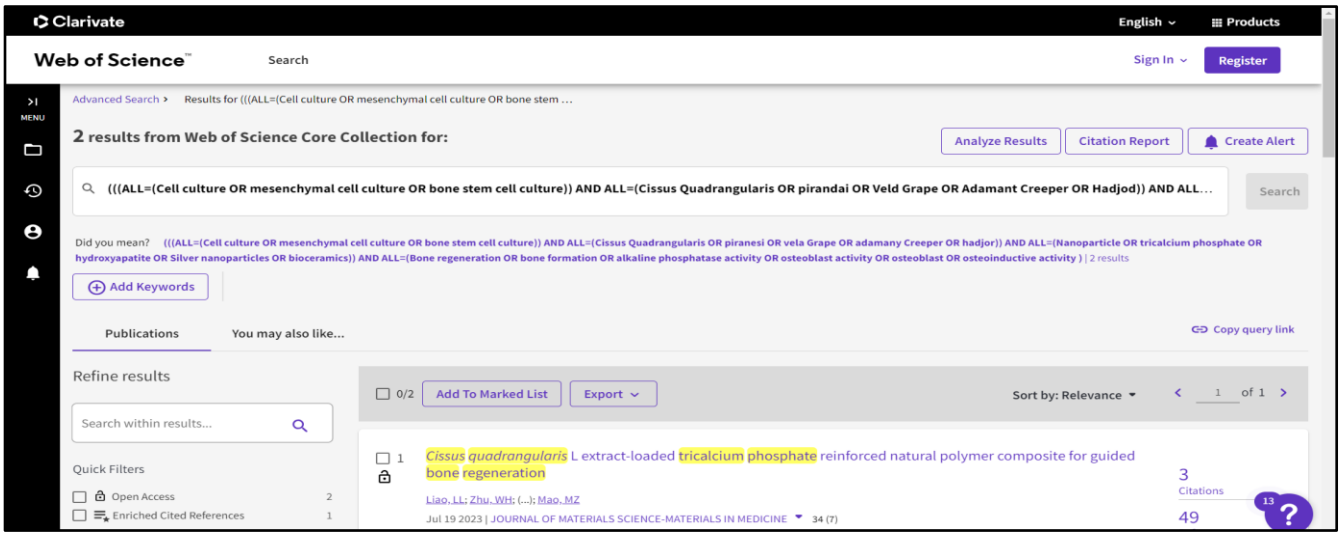


Figure 1 (a-d) . The search in different databases

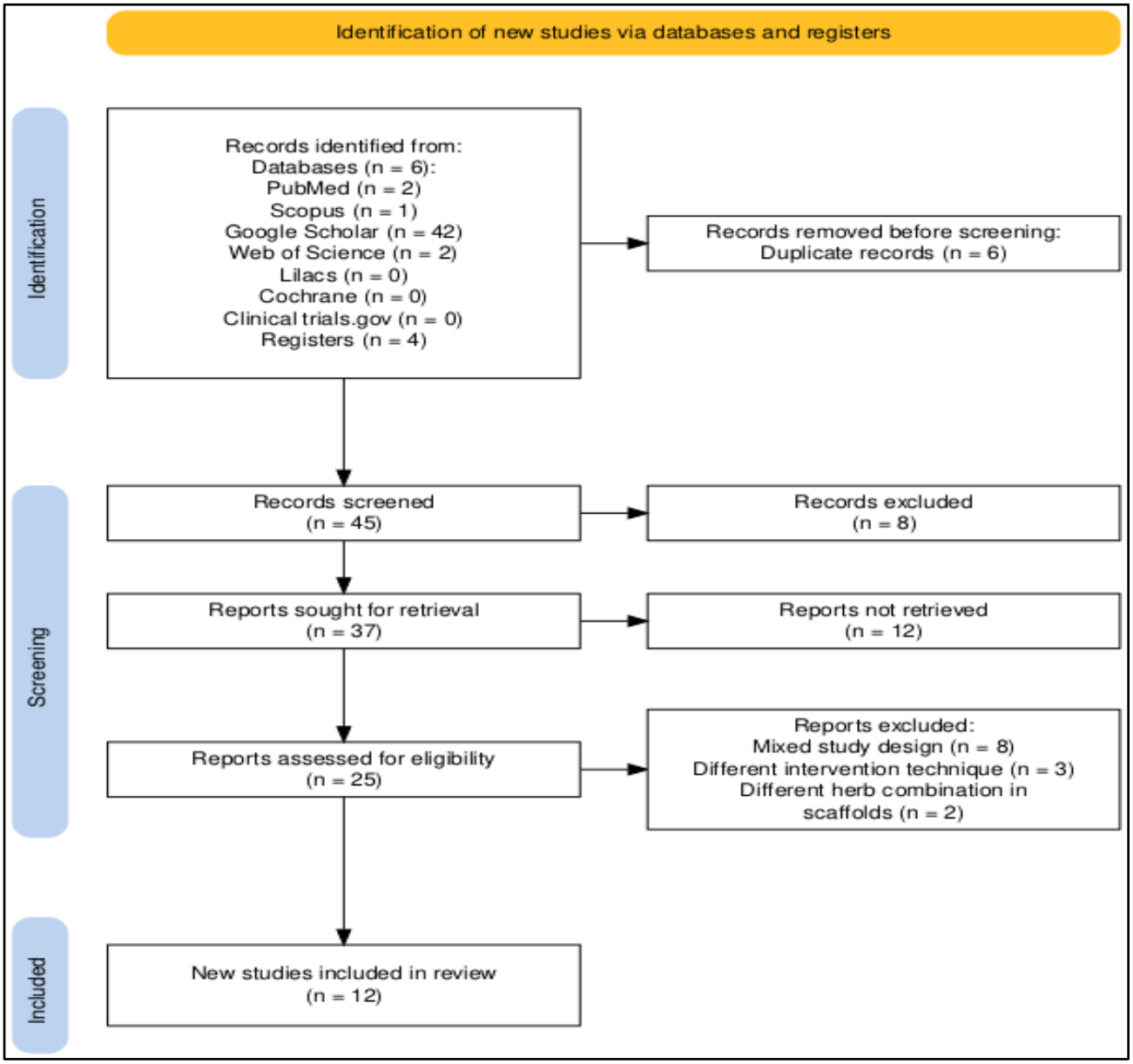


Figure 2. Prisma Flowchart for included studies

Using the databases Open Grey and GreyNet International, a search for grey literature was conducted in order to locate additional research that might be included in the review. References to the studies that were part of the review were also looked through in order to locate any research that might be included in the process of review.

The search was conducted independently by the two writers. First, a screening procedure was used to determine whether the article titles that the inquiry had turned up were eligible. Before beginning the qualitative investigation, we looked for and evaluated pertinent full-text studies. To discover if there was any more study that the initial search had missed, we browsed the references of the listed papers. A third reviewer was consulted multiple times during the search and review process in order to settle disputes that came up amongst the principal reviewers..

Data extraction

Two reviewers were needed for the data retrieval process. The following data was extracted and input into an already-prepared Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) from the studies that met the inclusion and eligibility requirements: This research includes the following: the number of samples, the degree of evidence, the intervention and control group, the first author, the nation, the year the study was published, the study design, the inclusion and exclusion criteria, and the key study outcomes. A third reviewer was used to verify the selection process. The final decision of inclusion into the article was decided by all the reviewers with voting process. Determined by the PRISMA flow diagram, Figure 2 illustrates the steps that go into selecting an article [17]. After the data extraction, each reviewer was assigned to specific number of articles to collect the data from the article manually.

Assessment of Risk of Bias

Specific risk of bias namely QUIN guidelines for in vitro studies were used to measure the risk of bias [18]. The requirements were broken down into 12 primary categories that namely

1. Clearly Stated Aims/Objectives
2. Detailed Explanation of Sample Size Calculation
3. Detailed Explanation of Sampling Technique
4. Details of Comparison Group
5. Detailed of Methodology
6. Operator Details
7. Randomization
8. Method of Measurement of Outcome
9. Outcome Assessor Details
10. Blinding
11. Statistical Analysis
12. Presentation of Results

Each study criterion was rated as 2 points for adequately specified; 1 point for inadequately specified, 0 points for not specified and NA for not applicable. The sum of all scores is assessed for risk of bias by a formula Sum of scores* 100/ 2* Number of applicable criteria.

The result of this above formula is categorised into low risk if its more than 70, moderate if its 50 - 70 and high risk if its less than 50. One reviewer assessed the possibility of bias, and the other cross-checked the results.

RESULTS

The search identified a total of 51 studies from various databases and registers. Six articles were removed due to duplication. Out of the remaining 45 studies screened, eight were excluded based on their abstracts as ineligible. From the 37 studies sought for full-text retrieval, 12 articles were excluded because their full texts could not be accessed. Consequently, 25 articles were reviewed in full text. Among these, eight were excluded due to containing both in vitro and in vivo experiments, three were removed because the intervention groups differed, and two were excluded due to the use of different herb combinations. After thorough screening, 12 studies were included in the review.

Out of the 12 studies included, 11 of them were published after 2016, only one was from 2009. Out of the 12 studies included, 10 were done on human cell culture and 2 were done on animal derived cell line which was specifically murine cell line. In the 10 human cell culture studies, 6 were done on mesenchymal cell culture, three were done on osteoblast cell line and one was done on human osteosarcoma cell line. Hydroxy apatite scaffolds were used in one study by Balaji et al, 3D printed β-Tri calcium Phosphate scaffolds were used in a study by Samuel F. Robertson.

There were different combinations of Cissus Quandrangularis were experimented in the studies. There were 5 one group intervention studies without comparison. In the other 7 studies Hydroxy apatite was used in the study Balaji et al, Hexane extract (HE), chloroform extract (CE), ethyl acetate extract (EE), methanol extract (ME) and aqueous extract was used in the study by Praseetha Nair et al. Europium-Monetite calcium phosphate complex was used in the experiment in the study by Niditha Suresh et al. poly ε-caprolactone scaffold and graphene oxide were used in a study by Shivaji Kashte et al. In the other 3 studies, different concentration of Cissus Quandrangularis extracts were compared.

The main test used in the included studies for bone regeneration was alkaline phosphatase activity. This was used in 9 out of 12 included studies.The enzyme alkaline phosphatase, which is involved in dephosphorylation processes, can be measured using a biochemical assay called alkaline phosphatase activity (ALP activity). It is frequently employed as a marker for mineralization and cell differentiation. In the other studies Alizarin Red Staining or sirius red staining was used to assess bone regeneration. It is important to note that Alizarin red staining is used in detecting calcium deposits in tissues whereas Sirius red staining was used assess collagen fibres. One study by Lele Liao et al, osteogenic marker genes Runt-related transcription factor, Vascular Endothelial Growth Factor and Osteocalcin were done to assess osteogenic activity.

Cissus Quandrangularis has been showed to increase periodontal tissue regeneration when its added in HA and tendon scaffolds. When it was added along with natural polymer in 3D printed β-Tri calcium Phosphate scaffolds, it increased the proliferation and alkaline phosphatase expression. When CQ was incorporated into nanovesicles, it was used as plant-derived extracellular vesicles (PDEVs) for bone regeneration and local drug delivery system in a study by Ritu Gupta. When CQ based Europium-Monetite composite was assessed for alkaline phosphatase activity on MG-63 cell line, it showed 10% higher ALP activity than that of the control (Monetite) in a study by Niditha Suresh et al. When CQ was mixed with a poly ε-caprolactone and graphene oxide scaffold it showed that its synergistic effect tenhanced osteoblastic differentiation, osteoconduction and osteoinduction potential of scaffolds in a study by Shivaji Kashte et al. When CQ based natural fibre was assessed on murine bone marrow Mesenchymal Cells, it increased the ALP activity in a study by Praseetha Nair et al.

The hexane extract and aqueous extract form of CQ proved to have strong osteogenic potential in a study by Praseetha Nair et al. When different concentrations, 300 µg/mL had the strong osteogenic potential compared to 100 and 200 µg/mL in the bone marrow mesenchymal cells. Also in a study by Parvathi K et al, ALP activity was measured in a herbal fiber sheet containing high weight percentages of CQ in 20, 40, and 60 wt% in poly (L-lactic acid) (PLLA). Although it has been demonstrated that the 40% CQ has substantial ALP activity, the 60% sheet has much lower cell survival after 14 days and should not be employed. When CQ was used in different concentration on the murine pre-osteoblast cell line, 200 mg/ml CQ had strong osteoblast differentiation in a study by Raazia Tasadduq et al. (Table 1)

QUIN guidelines showed that 11 of the included studies had low risk of bias and one study had unclear/ moderate risk of bias. (Table 2)

Table 1. Main characteristics of the included studies

S.No.	Author	Year	Stem Cell culture/ Scaffolds	Intervention groups	Test used	Results
1	Balaji Ganesh et al, Cureus [19]	2024	HA scaffolds in Mesenchymal cell culture	Group I – HA Group II - HA + CQ, Group III - HA + TENDON, Group IV- HA + CQ + TENDON	Differentiation analysis, and tenogenesis – Sirius red staining	Adding CQ significantly boosts the tenogenic potential of Extra cellular matrix /HA scaffolds, making them promising for periodontal tissue regeneration.
2	Samuel F. Robertson and Susmita Bose. Journal of the Mechanical Behavior of Biomedical Material [20]	2020	3D printed β-Tri calcium Phosphate scaffolds in Osteoblast cell cultures	natural polymer/drug coating of polydopamine and Cissus Quadrangularis extract	Alkaline phosphatase activity	Polydopamine in osteoblast cultures increased proliferation and alkaline phosphatase expression under dynamic flow, with further enhancement from Cissus Quadrangularis extract.
3	Praseetha Nair et al. Journal of Biosciences [21]	2021	Human Osteosarcoma cells	CQ stem based Hexane extract (HE), chloroform extract (CE), ethyl acetate extract (EE), methanol extract (ME) and aqueous extract (WE)	Alizarin Red Staining and Alkaline Phosphatase activity	The early bone marker alkaline phosphatase (ALP) confirms the strong osteogenic potential of HE and WE extracts compared to others, highlighting their rich content of bone-regenerative phytochemicals
4	Bhagath Kumar Potu et al. CLINICAL SCIENCE		Bone marrow mesenchymal stem cells	100, 200, and 300 µg/mL petroleum ether extract of Cissus quadrangularis and a control group	Alkaline phosphatase activity	Treatment with 100, 200, or 300 µg/mL petroleum ether extract of Cissus quadrangularis promoted differentiation of marrow mesenchymal stem cells. The 300 µg/mL concentration also enhanced cell proliferation

5	Sedef Tamburaci et al. Journal of Bioactive and Compatible Polymers [23]	2018	Osteoblast cell culture	Cissus quadrangularis with chitosan/Na-carboxymethyl cellulose blend scaffold	In vitro mineralization with von Kossa and Alizarin red staining and Alkaline phosphatase activity	A Cissus quadrangularis–loaded scaffold made from chitosan, and Na-carboxymethyl cellulose shows promise as a biomaterial for bone tissue engineering.
6	Lele Liao et al. Journal of Materials Science: Materials in Medicine [24]	2023	Human bone marrow mesenchymal stem cells	CQ extract with gelatin (Gel) and pectin (Pec) polymers collective through β- tricalcium phosphate (β-TCP) bioceramic	Osteogenic marker genes Runt-related transcription factor (RUNx), Osteocalcin (OCN), and Vascular Endothelial Growth Factor (VEGF); the mRNA levels were observed by utilizing Real-Time Polymerase Chain Reaction (RT-PCR)	CQ-loaded β-TCP/Gel-Pec composites are promising biomaterials for bone tissue repair and regeneration.
7	Ritu Gupta et al. Journal of Functional Biomaterials [25]	2023	Human-derived mesenchymal stem cells	CQ derived exosome-like nanovesicles	Alkaline phosphatase activity	An in vitro tissue culture system enables rapid, sterile growth and a reliable supply of plant-derived extracellular vesicles (PDEVs) for drug delivery applications.
8	Niditha Suresh and Karthikeyan. Cureus [26]	2024	MG-63 cell line	CQ based Europium-Monetite composite and Control - Monetite	Alkaline phosphatase activity	The osteogenic potential of the CQ-based Europium-Monetite calcium phosphate complex, as measured by ALP activity, calcium mineralization, and collagen staining, was 10% greater than that of the Monetite control.
9	Shivaji Kashte et al. Journal of Bioactive and Compatible Polymers [27]	2020	Human umbilical cord Wharton's jelly-derived mesenchymal stem cells	The modified PCL-GO-CQ scaffold was compared with plain poly ε-caprolactone scaffold and poly ε-caprolactone coated only with graphene oxide	Alizarin Red S staining	GO and CQ callus extract endowed the scaffold with osteoinductive properties, enabling hUCMSCs to differentiate into osteoblasts without additional media, growth factors, or external stimuli.

10	Parvathi K et al. International Journal of Biological Macromolecules [28]	2017	Mesenchymal stem cells	Group I – Poly fibres containing 20wt CQ Group II – Poly fibres containing 40wt CQ Group III - Poly fibres containing 60wt CQ	Alkaline phosphatase activity	Mesenchymal stem cells differentiated into osteoblasts without osteogenic supplements, demonstrating the scaffold's osteoinductive capability. The herbal sheet also promoted mineralization after 14 days in simulated body fluid.
11	Raazia Tasadduq et al. Journal of Cellular Physiology [29]	2016	Murine pre-osteoblast cell line, MC3T3-E1	Group I - CQ-E 200 mg/m, Group II – CQ-E 100 mg/m, Group III – CQ-E 50 mg/m, Group IV – CQ-E 25 mg/m, Group V – CQ-E 10 mg/m, Group VI – CQ-E 1 mg/m, Group VII – CQ-E 0.1 mg/m Group VIII - Dimethyl sulfoxide (DMSO) – control	Alkaline phosphatase activity	Cell proliferation significantly increased with lower concentrations of CQ-E. CQ-E also enhanced osteoblast differentiation, as shown by higher ALP activity and extracellular matrix mineralization compared to controls, indicating that lower CQ-E concentrations have anabolic and osteogenic effects
12	Praseetha R. Nair et al. Cellulose [30]	2021	Murine Bone marrow Mesenchymal Cells	Natural fiber derived from Cissus quadrangularis (CQ) stem	Alkaline phosphatase activity	The time-dependent ALP activity in BMC confirms osteoblast formation, making CQF a promising natural osteoinductive substrate for bone tissue engineering.

Table 2. Risk of Bias of the included studies by QUIN guidelines

S. No.	Study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	ROB
1	Balaji Ganesh et al, 2024	2	2	1	2	2	1	2	1	1	0	1	2	Low
2	Samuel F. Robertson and Susmita Bose. 2020	2	1	1	2	2	0	2	1	2	1	1	2	Low
3	Praseetha Nair et al. 2021	2	2	2	2	2	0	2	2	2	1	1	2	Low
4	Bhagath Kumar Potu et al. 2009	2	2	2	2	2	1	1	2	2	1	2	1	Low
5	Sedef Tamburaci et al. 2018	2	1	1	2	1	1	1	2	2	1	1	2	Low
6	Lele Liao et al. 2023	2	1	0	0	2	2	2	1	2	2	2	2	Low
7	Ritu Gupta et al. 2023	2	2	2	2	2	2	0	1	0	2	1	1	Low
8	Niditha Suresh and Karthikeyan. 2024	2	2	0	0	2	2	1	1	1	1	2	2	Medium
9	Shivaji Kashte et al. 2020	2	2	0	2	1	2	1	2	2	1	1	2	Low
10	Parvathi K et al. 2017	2	2	1	1	2	1	1	2	1	1	2	2	Low
11	RAAZIA TASADDUQ et al. 2016	2	2	2	2	2	1	2	1	2	0	2	2	Low
12	Praseetha R. Nair et al. 2021	2	2	2	0	2	1	2	1	2	0	1	2	Low

DISCUSSION

Human bone is made up of a mineralized organic matrix and various bone cells. Osteoblasts, which are mature bone cells, play a crucial role in forming the organic matrix and managing the mineralization of bone [31]. When a bone defect is too large to heal on its own, advanced therapeutic techniques are necessary to stimulate new bone growth. Osteogenesis, or bone formation, is a complex process that begins with the proliferation of mesenchymal stem cells, which then differentiate into pre-osteoblasts [32,33].

Next, these pre-osteoblasts mature into osteoblasts that produce type I collagen, the primary protein in the organic bone matrix or osteoid, accounting for approximately 90% of it. The active osteoblasts secrete large amounts of alkaline phosphatase, an enzyme that facilitates the deposition of minerals[34]. Calcium hydroxyapatite, which constitutes about 70% of bone mass, crystallizes within the collagen network, making the bone hard and capable of withstanding external forces while supporting the body and protecting internal organs. The rate of bone formation is influenced by the proliferation and activity of osteoblasts, and efficient bone repair depends on accelerated osteoblast growth [35].

In osteoporosis, bone mass decreases due to an imbalance where bone resorption outpaces formation. Key risk factors include aging, estrogen deficiency in postmenopausal women, decreased testosterone in men, smoking, low body weight, poor nutrition, alcohol use, and exposure to heavy metals like cadmium [36,37]. Bone marrow mesenchymal stem cells are the source of osteoblasts, and high bone turnover that results in bone loss is characteristic of osteoporosis. It is projected that by 2050, more than half of osteoporotic fractures will occur in Asia. Plant-based treatments may offer an affordable alternative for managing this condition [38].

Natural bioactive compounds have been used in medicine for decades and have proven highly beneficial in treating and preventing diseases. Cissus quadrangularis L. has traditionally been used for treating fractures, scurvy, tumors, hemorrhoids, peptic ulcers, menstrual disorders, and leucorrhea. It is also commonly used as a dietary supplement [39,40]. The bioactive compounds in CQ, such as glycosides, polyphenols, Vitamin C, and β-sitosterol, are believed to have antiulcer effects and enhance healing by releasing polyamines [21,41].

Research in recent years has explored the use of CQ's bioactive compounds in biomaterials for biomedical applications. For example, Tamburaci et al. previously investigated a scaffold made from chitosan and Na-carboxymethyl cellulose loaded with CQ extract, which showed osteoinductive properties and potential for bone tissue regeneration. Similarly, the construction of osteoinductive scaffolds, such as electrospun polycaprolactone scaffolds combined with CQ extract and graphene oxide, has been explored for bone repair [23,42].

In the present systematic review, 10 human and 2 animals cells were studied for bone regeneration in which C.quandrangularis has showed positive results. This showed that whether it is human or animal cell culture, bone regeneration was evident. Similarly, a study by Suganya et al in which polymeric nanofibrous scaffolds with CQ extract are assessed for bone regeneration, it showed potential biocomposite material [43].

Alkaline Phosphatase (ALP) test was done in most of the included studies. This served as the main biological markers. It is involved in the mineralization process, which is essential for healthy bone growth and repair. These are produced by bone-forming cells called osteoblasts, ALP helps integrate calcium and phosphate into the bone matrix, which is vital for bone strength and proper mineralization [44]. High levels of ALP typically indicate that bone formation is actively taking place and can be a sign that bone repair processes are working effectively. Clinically, measuring ALP levels helps diagnose conditions like osteoporosis and Paget's disease [45,46]. This ALP marker was used in many studies for assessing invivo, invitro bone repair and healing [47–49].

One interesting included study by Ritu Gupta showed that CQ was incorporated into nanovesicles, it was used as plant-derived extracellular vesicles (PDEVs) for bone regeneration and local drug delivery system. Similar herbal PDEV were used in different studies [50–53].

This present review also had few limitations. The review only included qualitative analysis of the studies. Another is it only included invitro studies and invivo studies were excluded. Quantitative analysis or meta analysis could not be done because of lack of homogeneity in the included studies. Future review should include more different type of studies with meta analysis.

CONCLUSION

In summary, this systematic review consolidates findings from twelve studies that evaluated the bone

regenerative potential of Cissus quadrangularis-infused scaffolds using both animal and human cell cultures. The techniques employed, such as alkaline phosphatase (ALP) activity assays and alizarin red staining, consistently indicated positive effects on bone regeneration across all studies.

These results suggest that Cissus quadrangularis holds significant promise as an active component in scaffolds for improving bone repair and regeneration. However, it is important to acknowledge that this review is limited by its focus exclusively on in vitro studies. While these studies offer useful preliminary data on the regenerative potential of Cissus quadrangularis-infused scaffolds, they do not fully capture the complexities of biological systems in living organisms. Future research should address this gap by conducting well-designed in vivo studies to assess the effectiveness and safety of these scaffolds in real-world settings. Additionally, exploring the long-term impacts and interactions of Cissus quadrangularis with various biological systems will be critical for its advancement in bone regenerative treatments.

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ORIGINAL ARTICALE

FABRICATION AND CHARACTERIZATION OF VITAMIN C CONJUGATED CISSUS QUADRANGULARIS MEMBRANE FOR GUIDED TISSUE REGENERATION - AN IN VITRO STUDY

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ABSTRACT

Background: Cissus quadrangularis has gained attention for its rich content of bioactive compounds, including flavonoids, carotenoids, and polyphenols, notably Vitamin C.These compounds are recognized for their antioxidant, anti-inflammatory, and tissue reparative properties, may provide the membrane more bioactivity, which would encourage cell division and proliferation.This main aim of this study is the production of vitamin C membrane impregnated with Cissus quadrangularis for soft tissue regeneration.

Materials and Methods: To prepare the vitamin C membrane with the impregnation of Cissus quadrangularis (CQ) , 1 gram of CQ was dissolved in 10 mL of distilled water and heated at 80°C for 24 hours. Following membrane formation, FTIR (Fourier Transform InfraRed Spectroscopy), contact angle, tensile strength testing and antimicrobial activity was compared between the CAR control group membrane and the test group membrane which contains CAR with the impregnation of vitamin C.

Results: The tensile strength of the test group was better than the control group. The test group showed the tensile strength of 23.86 N whereas the control group showed 27.99 N. CAR membrane had a contact angle of 81.88 degrees. This implies lower wettability. Whereas the cissus quadrangularis and vitamin C incorporated membrane had a contact angle of 49.92 degrees. The test group showed better wettability, tensile strength and anti microbial activity as compared to the control group.

Conclusions: The present study showed that CAR membranes with the impregnation of Cissus quadrangularis showed increase in hydrophilicity. The mechanical property was increased and the antimicrobial activity showed an enhanced zone of inhibition. The membrane can serve multipurpose dental applications and can be incorporated in various periodontal procedures for guided tissue regeneration.

Keywords: vitamin C, Cissus quadrangularis, soft tissue regeneration, Periodontal regeneration

INTRODUCTION

Gingivitis and periodontitis are two of the periodontal disorders, which are arguably the most prevalent illnesses in the world. Since periodontitis is the leading cause of tooth loss in adults worldwide, these people are susceptible to edentulism, masticatory dysfunction, and multiple tooth loss, which can negatively impact their nutrition, quality of life, and self-esteem in addition to having significant socioeconomic and medical costs. It is possible to prevent, diagnose, treat, and control periodontitis with the help of proper medical care and long-term secondary prevention.[1]While periodontal flap surgeries and conventional nonsurgical therapy are effective in slowing the progression of periodontal disease. Guided bone/tissue regeneration (GBR/GTR) techniques are surgical techniques used to regenerate lost bone/tissue and are accepted treatment modalities for alveolar bone augmentation and the reconstruction of lost periodontal structures in periodontal defects.[2] Barrier membranes are used in these techniques to impede the ingrowth of soft tissue into defect sites and to promote periodontal tissue or bone regeneration. Various biomaterials are used in guided tissue regeneration. Nowadays naturally occurring plant-based chemicals with bioactive and regenerative qualities are used as strategy to treat periodontal defects.[3]

The medicinal plant Cissus quadrangularis (CQ), which has long been used in herbal therapy, has shown great promise in accelerating the repair of soft tissues and bones. According to reports, it possesses anabolic, analgesic, and anti-inflammatory properties. It can also improve tissue healing and collagen formation. It has been demonstrated that CQ's active ingredients, which include flavonoids, polyphenols, and triterpenoids, contribute to its regenerative qualities, which makes it a desirable option for soft tissue repair applications. Its bioavailability and the requirement for improved delivery systems that may more precisely target and promote tissue regeneration, however, frequently restrict its clinical use.[4] Vitamin C (ascorbic acid), a well-known antioxidant and cofactor in collagen synthesis, plays a crucial role in the formation of collagen fibers during tissue repair and regeneration. It is essential for stabilizing collagen and promoting cellular functions that contribute to wound healing. Given its pivotal role in tissue regeneration, the conjugation of CQ with Vitamin C offers a novel approach to improving the regenerative potential of CQ, enhancing its efficacy in soft tissue repair.[5]

This research aims to explore the development of a Vitamin C conjugated Cissus quadrangularis membrane as a bioactive material for soft tissue regeneration. By harnessing the synergistic effects of CQ and Vitamin C, we seek to create a membrane that not only supports cellular attachment and proliferation but also stimulates the regenerative processes necessary for the healing of soft tissues.[6] The conjugation of Vitamin C to CQ is expected to improve the stability and bioactivity of the composite, while also enhancing the therapeutic potential for faster and more effective tissue regeneration. The main aim of this study is to fabricate and characterize Vitamin C conjugated Cissus quadrangularis membrane for guided tissue regeneration.

MATERIALS AND METHODS

MEMBRANE PREPARATION

To prepare the carrageenan membrane with the impregnation of Cissus quadrangularis (CQ) and vitamin C, 1 gram of CQ was dissolved in 10 mL of distilled water and heated at 80°C for 24 hours. This process facilitated the extraction of bioactive compounds from the plant material. After heating, the solution was filtered through Whatman filter paper to obtain a clear CQ extract, which was set aside for later incorporation into the CAR matrix. Simultaneously, a CAR solution was prepared by dissolving 5 grams of carrageenan in 100 mL of distilled water, and this mixture was kept overnight to ensure complete dissolution. Once fully dissolved, the carrageenan solution was divided into two equal parts. The first 50 mL served as the base membrane, consisting of pure carrageenan. To the second 50 mL, 50 mg of vitamin C was added, and the mixture was stirred for 5 hours to achieve a uniform dispersion of vitamin C. This vitamin C impregnated carrageenan solution was then poured into a petri dish and placed in a deep freezer at -20°C for 24 hours to promote solidification. After freezing, the membranes were lyophilized to remove any residual moisture, yielding stable CAR membranes ready for guided tissue regeneration applications. The materials were divided into two groups: The control group consists of carrageenan(CAR) and the test group consists of the addition of Cissus quadrangularis and vitamin C to carrageenan (CAR+vitamin C).

CHARACTERISATION OF THE MEMBRANES

The membranes were cut into smaller samples and tested using different parameters. FTIR (Fourier Transform InfraRed Spectroscopy), contact angle, tensile strength testing and antimicrobial activity was compared between the CAR control group membrane and the test group membrane which contains CAR with the impregnation of cissus quadrangularis and vitamin C.

RESULTS

Fourier transform infrared spectroscopy (FT-IR) was

measured with the help of Perkin Elmer. This FT-IR comparison shows spectra for a CAR (carrageenan) control group sample and a CAR+ vitamin C test group membrane. When Vitamin C is present, the CAR+ vitamin C test group (blue) spectrum exhibits more peaks or shifts than the CAR (yellow) spectrum. These variations most likely show that vitamin C has been added to the CAR matrix. The addition of vitamin C is confirmed by the unique peaks between 1000 and 800 cm that are present in the CAR+ vitamin C spectrum but lacking in the CAR spectrum. These peaks are probably caused by the Amide and carboxyl groups in vitamin C indicates the formation of material. Amide and carboxyl groups in CAR revealed that the amide I and amide Il bands, which are typically located at cm-' and cm, respectively, are typical FT-IR peaks for CAR. Interactions between Hydroxyl Groups revealed that possible hydrogen bonding interactions between CAR and vitamin C may be indicated by shifts or variations in intensity around the 3200–3600 cm-1 area (hydroxyl stretching) (Figure 1).

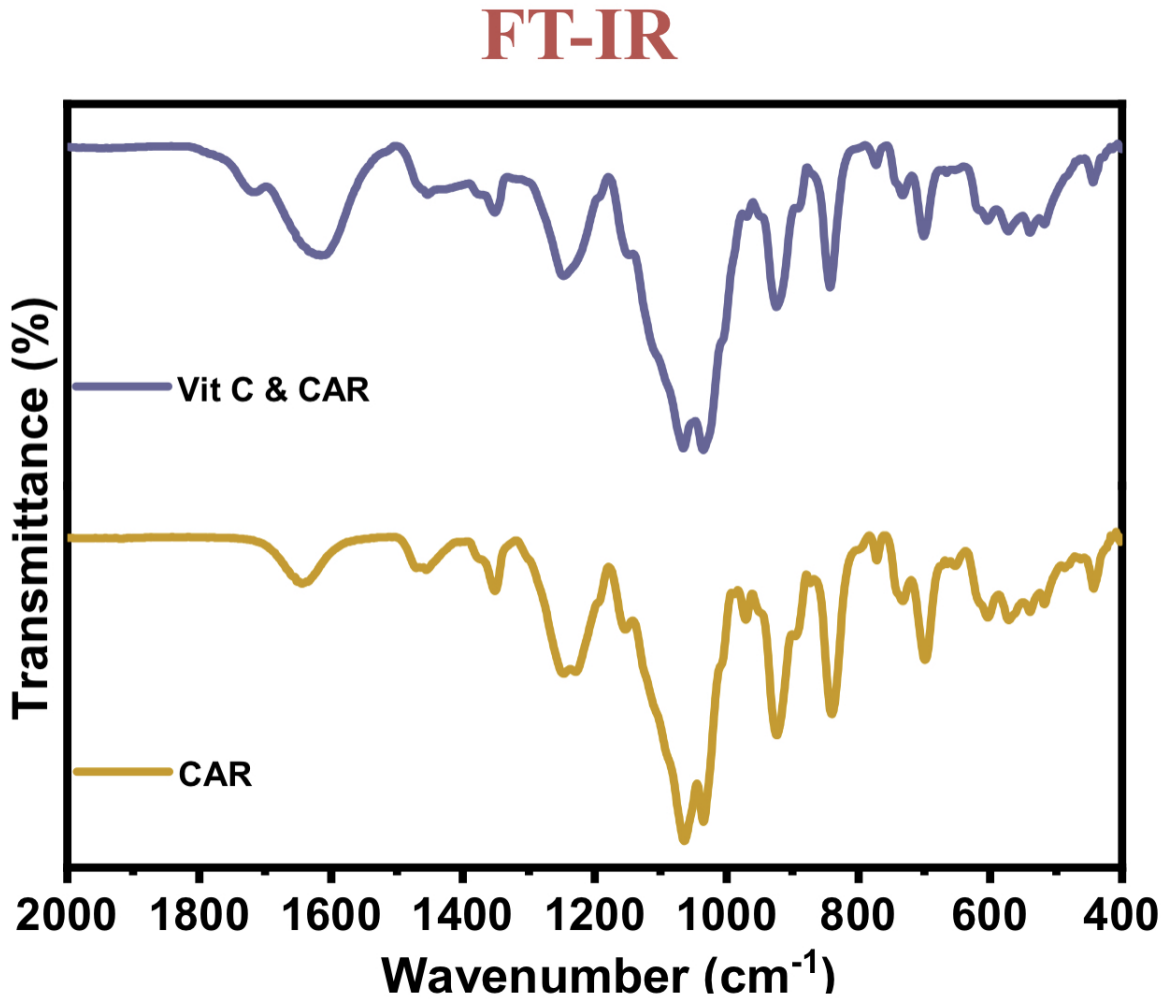
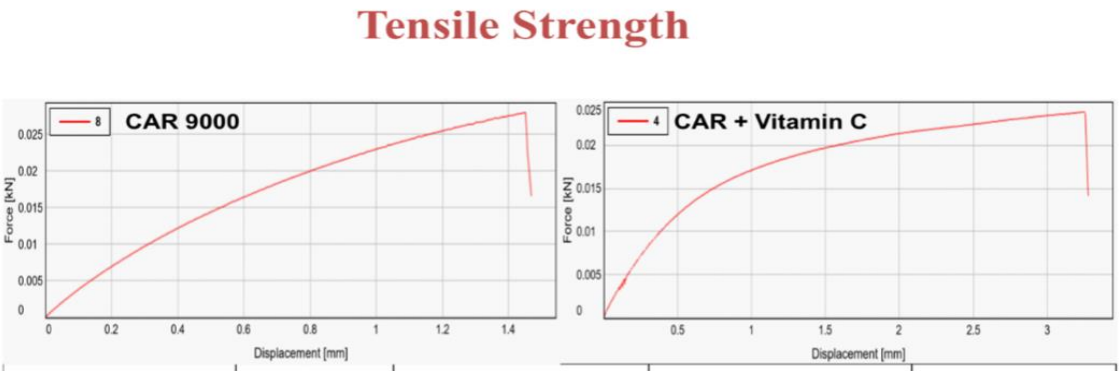


Figure 1. shows the Fourier transform infrared spectroscopy

Tensile strength was measured in newtons and it was tested using the INSTRON 3000 universal testing machine. The tensile stress for 17.02 MPa with 8.15% tensile strain at break and 17.01 MPa tensile stress at break whereas the addition of Cissus quadrangularis to vitamin C (test group) showed a tensile stress of was 19.99 MPa, with 3.63% tensile strain at break and 19.99 MPa tensile stress at break. The tensile strength of CAR (control group)was 23.86 N whereas the CAR + vitamin C (test group) showed 27.99 N. This shows an enhanced property of tensile strength in the membrane that was incorporated with vitamin C and cissus quadrangularis (Figure 2).



Materials Name	Maximum Force [N]	Tensile stress at Tensile strength [MPa]	Tensile strain (Displacement) at Break (Standard) [%]	Tensile stress at Break (Standard) [MPa]
Vitamin C	27.99	19.99	3.63	19.99
CAR	23.86	17.02	8.15	17.01

Figure 2. represents the Tensile strength

Contact angle was measured using an ossila goniometer. Control CAR membrane had a contact angle of 81.88 degrees. This implies lower wettability. Whereas the cissus quadrangularis and vitamin C incorporated membrane had a contact angle of 49.92 degrees. This suggests higher wettability of the test group membrane. Thus the test group membrane had a good wettability property (Figure 3).

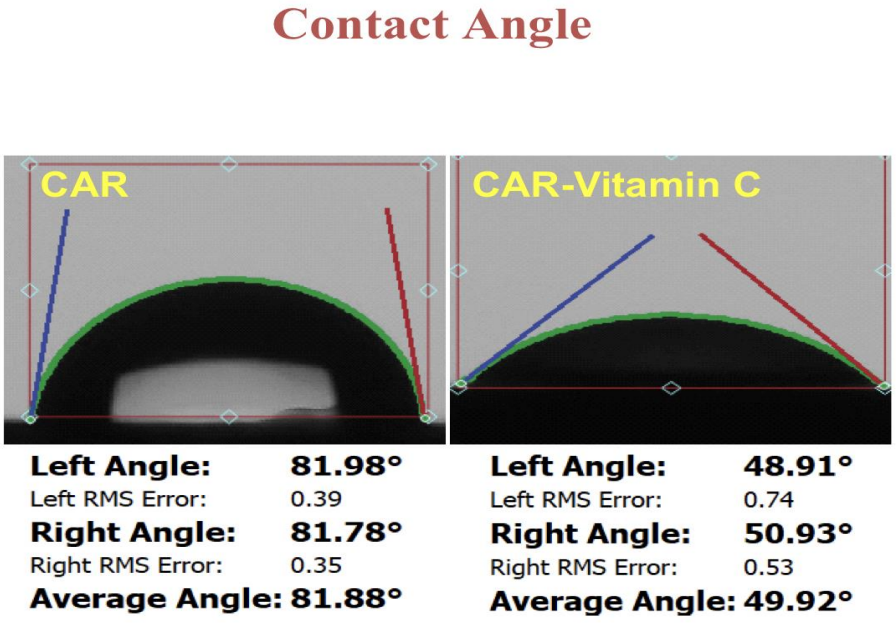


Figure 3. shows the difference in contact angle

The zone of diameter of S. mutans when incorporated with vitamin C and cissus quadrangularis was 13 mm whereas in the control group with CAR was 4 mm. This shows that better antimicrobial activity was found in the test group membrane than the control group membrane (Figure 4).

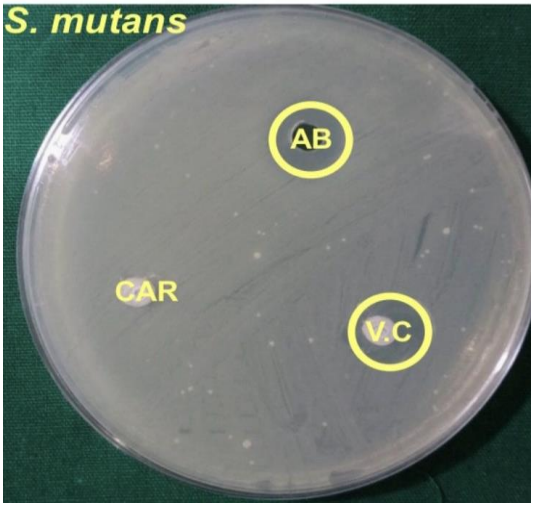


Figure 4. Shows antibacterial activity

DISCUSSION

In this study, we have successfully fabricated and characterized a novel vitamin C conjugated Cissus quadrangularis membrane (Vitamin C-CQ membrane) designed for guided tissue regeneration (GTR), with a specific focus on its in vitro performance.[7] The purpose of this research was to explore the potential benefits of combining the bioactive properties of Cissus quadrangularis, a well-known plant with healing properties, with vitamin C, an essential nutrient known for its antioxidant and collagen synthesis-promoting effects. The resultant membrane was assessed for its physical, chemical, and biological properties to evaluate its suitability for application in tissue regeneration, particularly in bone and soft tissue healing**.**

The healing of musculoskeletal tissues, such as bone, tendons, and ligaments, is dependent on the capacity of collagen synthesis and cross-linking.[8] Poorly developed extracellular matrices derived from collagen can lead to inadequate tissue structures and biomechanical strength, which can result in unsatisfactory outcomes and an increased risk for reinjuries.[9] Basic science investigations on the biochemical pathways after a musculoskeletal injury have suggested that vitamin C, also known as ascorbic acid, may enhance collagen synthesis and soft tissue healing. Another study ascertained the innate early biomineralization proficiency and sufficient mechanical stability complemented with controlled degradation of the chitosan–collagen scaffold; hence, could be proposed as a good candidate for regenerative bone tissue engineering. Studies have shown that Vitamin C plays a crucial role in collagen synthesis, a fundamental process in tissue repair. In a previous study , it was concluded that doping HA-based hydrogel scaffold with the natural product CQ has increased the tenogenic potential. When the tenogenesis capacity is increased, it also plays a significant role in orienting the collagen fibers, thereby inducing more tensile strength.[10] By facilitating collagen production and deposition, Vitamin C-conjugated membranes promote cellular proliferation and extracellular matrix formation, essential for restoring tissue integrity. In this study, Vitamin C incorporated carrageenan membrane showed increased tensile strength ensuring better regenerative properties of guided tissue regenerated (GTR) membrane.[11]

Ensuring the biocompatibility and long-term stability of the membranes is crucial for their clinical translation.The surface functionalization of Cissus quadrangularis with vitamin C was confirmed through Fourier-transform infrared (FTIR) spectroscopy, which showed characteristic peaks corresponding to both vitamin C and Cissus quadrangularis.[12] This supports the notion that vitamin C was successfully incorporated into the membrane and could potentially impart additional benefits, such as antioxidants and collagen biosynthesis, critical for tissue repair processes. Hydrophilic properties have a water contact angle (WCA) of less than 90°. From this study , the sample with vitamin C can be said to have the better hydrophilicity with the lower contact angle of 49.92°.[13] The higher contact angle value would reflect a better surface solid hydrophobicity. In a previous study by Mat Yassin et al. (2022) produced a carrageenan-based biofilm with surface hydrophobicity ranging from 81 to 107°. The films were blended with lipid which was extracted from *cholera vulgaris.* In this work, the hydrophobicity reduced with the addition of vitamin C. This is probably due to vitamin C being highly soluble in

water.[14]

The traditional medicinal practice of Ayurveda has long utilized CQ to address a spectrum of diseases and ailments.[15] Its recognized osteogenic properties prompted an exploration into its potential application for periodontitis.[16] Our findings confirm the antibacterial properties of CQ, shedding light on its potential therapeutic role in combating periodontal infections.In a previous study , results demonstrated significant antibacterial properties of CQ aqueous and ethanolic extracts against *Porphyromonas gingivalis*. This study shows S. mutans when incorporated with vitamin C and cissus quadrangularis had better antimicrobial activity when compared to CAR.

A more thorough assessment of the vitamin C-carrageenan membrane antibacterial efficacy would come from testing against other bacteria, particularly those that are frequently linked to periodontal illnesses or pertinent to the intended use (e.g. P. gingivalis, Actinobacillus). Because the study was conducted in a controlled in vitro setting, it might not accurately reflect situations in vivo.It will be necessary to test the material's regenerative capabilities using animal models. These limitations should be addressed in future studies to enhance the results and provide a more solid foundation for potential periodontal regenerative applications.

CONCLUSION

From this study we can conclude that, Cissus quadragularis conjugated vitamin C membrane shows high hydrophilicity and antibacterial activity along with high tensile strength when compared to pure carrageenan membrane. Therefore it can be used for guided tissue regeneration material in managing periodontitis patients .

DECLARATIONS

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No

Conflict of Interest

No

Support

No

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IN VIVO EVALUATION OF MICROBIAL CHANGES IN DELAYED VERSUS IMMEDIATE IMPLANT PLACEMENT

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ABSTRACT

Background: Peri-implant microbial colonisation is one of the most critical determinants of the success of osseointegration of implants and long-term outcomes. This in vivo study seeks to determine and compare qualitative and quantitative changes that occur in peri-implant microflora during immediate and delayed implant placement.

Materials and Methods: A total of 30 systemically healthy participants who needed single tooth extraction and implant placement were enrolled and equally allocated into two groups. Group A (immediate placement of an implant), and Group B – a delayed implant placement 12 weeks after surgery. Peri-implant sulcus was sampled at the start (pre-placing), the first month, and three months after placement. For this, sterile paper points were utilized. Cultivation and identification were done by culture-based methods together with colony-forming unit (CFU) counts for *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum*. Clinical measures such as plaque index and gingival index, and peri-implant probing depth were also documented.

Results: Group A exhibited a higher mean CFU count for P. gingivalis (2.8×10^5CFU/mL) than Group B (1.2×10^5CFU/mL). The S. mutans colonization at three months was comparable for both groups. There was a statistically significant ($p<0.05$) increase in anaerobic species in Group A. The clinical parameters did not exceed the limits in both groups. However, slightly better gingival scores and reduced inflammation were demonstrated in Group B.

Conclusion: One of the possible consequences of the immediate implant placement might be a higher level of colonization of pathogenic anaerobic bacteria during the preceding period of healing in comparison with delayed implant placement. Postponed placement may promote more optimal processes of soft tissue healing and microbial stabilization. Monitoring of per-implant microbial profiles is critical in the beginning stages of implant therapy.

Keywords: Immediate implant, delayed implant, peri-implant microbiota, *P. gingivalis*, microflora, in vivo study, CFU count.

1. INTRODUCTION

Dental implants have transformed restorative dentistry as a reliable and predictable therapy for replacing missing teeth. There has been a lot of interest and clinical debate about the timing of implant placement: immediate versus delayed.

Immediate implant placement implies the insertion of the implant fixture into the extraction socket during the same surgical visit, and the delayed placement is

performed after a healing period of several weeks to months after tooth extraction[1]. Each of these approaches has its merits and limitations in the aspects of esthetics, soft tissue healing, and preservation of peri-implant bone.

Establishment and stability of the peri-implant microbial environment are one of critical factors that determine the success of dental implants. The colonization of the implant surfaces by the oral microbial flora, in particular by pathogenic anaerobic microbes, can be the stimulus for the peri-implant inflammation and imperil osseointegration[2–4]. Studies have shown that the microbial pattern around the implant is similar to that observed in the natural periodontal sulcus and often dominated by *Porphyromonas gingivalis, Fusobacterium nucleatum, and Aggregatibacter actinomycetemcomitans*[5]. These bacteria are highly associated with peri-implantitis and implant failure[6].

Prompt implant insertion is beneficial in terms of retaining treatment duration and saving alveolar bone, yet, in compromising the surgical site and having poor soft-tissue coverage for the initial period of healing early on, there is the increased risk for microbial contamination[7]. On the other hand, delayed placement of the implant allows for initial soft and hard tissue healing, which may decrease microbial load and encode a more stable peri-implant situation[8,9].

With a large body of literature on implant microbiology, very few studies of a similar nature have been carried out in vivo to determine the microflora change associated with immediate and delayed implants. Insight into evidence of changes in the microbial dynamics in the implants installed with various intervals of time is essential to optimize the clinical protocols and enhance the long-term prognosis of implants. This research aims to assess and compare qualitative and quantitative alterations in implant microflora in immediate and delayed implant placement using the culture-based method of microbial analysis.

2. MATERIAL AND METHODS

This in vivo comparative study was conducted on 30 patients aged between 25 and 55 years, who required a single tooth replacement in the posterior region of the mandible or maxilla. All participants were systemically healthy, non-smokers, and demonstrated good oral hygiene.

Study Design and Group Allocation

Participants were randomly assigned to two groups of 15 patients each:

- Group A: Immediate implant placement immediately after tooth extraction.
- Group B: Delayed implant placement performed 12 weeks post-extraction after adequate soft tissue and bone healing.

Atraumatic extraction was performed with the use of periotomes and forceps to avoid damage to the surrounding bone. In Group A, implants were positioned in the fresh extraction socket in the same surgical visit. In Group B, the time to heal and therefore the time to the extraction was 3 months, and implants were placed after. All implants were inserted under local anesthesia, according to a standard surgical protocol. Bone grafts and membrane barriers were not used between the two implants to prevent confounding factors. The postoperative instructions and antibiotics for five days were given to the patients.

The peri-implant sulcular fluid was drawn using sterile paper points at three intervals of time. Baseline (pre-operative), 1 month, and 3 months after the implantation. Paper points were inserted into the peri-implant sulcus for 30 seconds prior to being put into sterile transport media right away.

Samples were grown on selective media to detect and count *Streptococcus mutans, Porphyromonas gingivalis, and Fusobacterium nucleatum*. Incubation was carried out under conditions lacking oxygen for 48-72 hours. CFUs per mL were calculated and recorded for every bacterium.

Some of the clinical measures, such as Plaque Index (PI), Gingival Index (GI), and peri-implant Probing Depth (PD), were recorded at baseline, after 1 month, and after 3 months. All clinical assessments were conducted by a calibrated examiner to eliminate variability.

SPSS version 25.0 statistical package was used to analyze the data. Intergroup comparisons were conducted with the aid of an independent t-test, while intra-group comparisons over the time points were analyzed with the aid of repeated measures ANOVA. Statistical significance was assumed at a p-value < 0.05.

3. RESULTS

A total of 30 patients (15 in each group) completed the study without any implant failure or postoperative complications. The demographic distribution was comparable between the two groups, with a mean age of 38.4 ± 6.2 years in Group A and 39.1 ± 5.8 years in Group B.

Microbial Analysis

The mean colony-forming units (CFU/mL) for *Porphyromonas gingivalis, Fusobacterium nucleatum,* and *Streptococcus mutans* at baseline, 1 month, and 3 months are shown in **Table 1**. At 1 month and 3 months, *P. gingivalis* and *F. nucleatum* levels were significantly higher in Group A (immediate placement) compared to Group B (delayed placement) ($p < 0.05$). *S. mutans* levels remained relatively stable across both groups with no statistically significant differences.

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Table 1. Mean CFU/mL of Key Microorganisms at Different Time Intervals in Both Groups

Bacterial Species	Time Point	Group A (Immediate)	Group B (Delayed)	p-value
P. gingivalis	Baseline	$1.1 \times 10^5 \pm 0.2$	$1.0 \times 10^5 \pm 0.3$	0.612
	1 Month	$2.4 \times 10^5 \pm 0.5$	$1.3 \times 10^5 \pm 0.4$	0.032*
	3 Months	$2.8 \times 10^5 \pm 0.4$	$1.2 \times 10^5 \pm 0.3$	0.018*
F. nucleatum	Baseline	$0.9 \times 10^5 \pm 0.1$	$0.8 \times 10^5 \pm 0.2$	0.408
	1 Month	$2.0 \times 10^5 \pm 0.3$	$1.0 \times 10^5 \pm 0.2$	0.029*
	3 Months	$2.5 \times 10^5 \pm 0.5$	$1.1 \times 10^5 \pm 0.3$	0.015*
S. mutans	Baseline	$1.3 \times 10^5 \pm 0.3$	$1.2 \times 10^5 \pm 0.4$	0.676
	1 Month	$1.4 \times 10^5 \pm 0.2$	$1.3 \times 10^5 \pm 0.3$	0.543
	3 Months	$1.4 \times 10^5 \pm 0.3$	$1.2 \times 10^5 \pm 0.2$	0.482

*Significant at $p < 0.05$

Clinical Parameters

Clinical parameters such as Plaque Index (PI), Gingival Index (GI), and Probing Depth (PD) are summarized in **Table 2.** At 3 months, Group B showed significantly lower GI and PD values compared to Group A ($p < 0.05$), while plaque scores remained statistically similar between the two groups.

Table 2. Comparison of Clinical Parameters Between Immediate and Delayed Implant Groups

Parameter	Time Point	Group A (Immediate)	Group B (Delayed)	p-value
Plaque Index	Baseline	0.82 ± 0.11	0.80 ± 0.13	0.743
	3 Months	0.95 ± 0.09	0.89 ± 0.08	0.356
Gingival Index	Baseline	1.02 ± 0.14	0.98 ± 0.12	0.624
	3 Months	1.24 ± 0.10	0.96 ± 0.11	0.041*
Probing Depth	Baseline	2.2 ± 0.3 mm	2.1 ± 0.4 mm	0.487
	3 Months	2.6 ± 0.4 mm	2.1 ± 0.3 mm	0.038*

*Significant at $p < 0.05$

As shown in **Table 1**, microbial colonization by anaerobic pathogens was significantly higher in the immediate implant group at both follow-up points. This was further supported by the clinical findings in **Table 2**, which indicated increased inflammation and probing depth in the same group.

4. DISCUSSION

The current in vivo study sought to assess and compare the peri-implant microbial changes between the immediate and delayed implant placement programs. The results indicated that there were significantly more colonization of the pathogenic anaerobic bacteria, such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, in the immediate implant placement compared to the delayed implant placement at 1 month and 3 months post-placement. Based on these findings, one can conclude on a greater number of microbes, which in turn may have led to the increased risk for peri-implant inflammation in the immediate implant protocols during the early healing phase.

The initial colonization of the implant surfaces by the bacteria from the oral cavity is, therefore, a key factor governing the sustained long-term success of osseointegration and implant stability[1]. Immediate implantation into fresh extraction sockets can lead to premature exposure to biofilms in the oral cavities before the soft tissue maturation, which will hence favor the propagation of periopathogenic species[2,3]. As opposed to it, delayed implant placement permits the surgical site initial healing, which possibly can reduce microbial contamination and enhance more favorable tissue integration[5,6].

Results of our study are consistent with earlier works that indicated increased numbers of anaerobic bacteria in immediately placed implants over delayed protocols[7,8]. For example, the increased prevalence of *P. gingivalis* and *F. nucleatum* in the crevicular fluid of immediate implants was observed significantly by Devides SL et al.[10], implying delayed healing responses and more susceptibility to microbes. The same trends were present in the present study, where the immediate group exhibited nearly double counts of the CFU of such pathogens compared to the delayed group, at the 3-month evaluation.

The fairly constant levels of Streptococcus mutans in both groups could mean that facultative anaerobes do not have significant changes based on implant timing. This observation corresponds to findings from Lee KH et al., who revealed that the early microbiota associated with implants is essentially commensal streptococci before the shift to anaerobic colonization[11].

Some of the clinical parameters, like Plaque Index, Gingival Index, and Probing Depth, are adjunct indicators of health status around implants. Our delayed group exhibited better gingival health and fewer records of probing depths after 3 months, and the same was consistent with fewer anaerobic bacterial counts. These clinical outcomes reflect the corresponding microbiological results and confirm a hypothesis that delayed implants constitute a more facilitative environment for early healing[12–14].

There are numerous mechanisms that could affect microbial colonization and peri-implant health, such as surgical technique, oral hygiene of patients, surface characteristics of implants, and perioperative care[15]. It is worth mentioning that in the current study, all implant plastic surgeries were performed under standardized protocols and with the same surgeon, meaning we have reduced operator-dependent variability. Limitations are the relatively short follow-up, as well as the use of culture-based microbiology, which could miss the unculturable species that some advanced molecular techniques could unveil[16,17].

However, clinically, the implication of this study is valid. The greater number of microbial challenges recorded in immediate implant placements calls for selective choice of patients, practices of high infection control measures, and postoperative monitoring. In active infection or poor quality of soft tissue, a delayed surgical approach could result in optimal outcomes.

Greater cohorts, longer durations of follow-up, and implementation of next-generation sequencing analysis, thus, can provide further information on the dynamics of the microbial population around implants in regard to implant timing and their positive or negative contribution to the health of peri-implant tissues[18].

5. CONCLUSION

Results of this in vivo study show that immediate implant placement entails a greater early colonization of anaerobic periopathogenic bacteria than as compared to delayed implant placement. Peri-implant tissues and microbial control were better during the first stages of healing for those with a delay in implants. Such results highlight the need for suitable case selection, microbial control, and rigid hygiene procedures during immediate implant surgeries to optimize clinical outcomes and long-term implant prosperity.

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 ARTICALE

A COMPARATIVE ANALYSIS ON EFFICACY OF HERBAL EXTRACTS AND CHLORHEXIDINE ON CLINICAL AND MICROBIOLOGICAL PARAMETERS IN CHRONIC PERIODONTITIS- A SYSTEMATIC REVIEW AND META-ANALYSIS

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ABSTRACT

Objective: To assess effect of herbal medicines compared to 0.12% chlorhexidine (CHX) in periodontitis

Materials and Methods: Review was adhered with Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines and registered in PROSPERO- CRD42024506918. From January 2000 to January 2025, research on the benefits of herbal medicines vs 0.12% CHX on periodontitis in terms of plaque index (PI), gingival index (GI), clinical attachment level (CAL), deep pocket (PPD), and colony forming unit (CFU) was found by searching electronic databases. Using Review Manager (RevMan) software version 5.3, the Cochrane risk of bias (ROB) -2 tool for randomized controlled trials (RCT) was employed to evaluate quality in all of its domains. The standardized mean difference (SMD) was used as a brief statistic measure with a random effect model ($p<0.05$).

Results: Meta-analysis was suitable for nine out of the fourteen studies that satisfied the criteria for selection in the qualitative synthesis. The quality evaluation determined that the risk of bias was moderate to low. A variety of herbs like Punica granatum, Herborals, Morus alba, Natural curcumin, Salvadora persica, Khadiradi vati, Dashansanskar churana, Neem and Apamarga, Ferula assa-foetida, Aloe vera, tea tree oil, Guava leaf extract, Ocimum sanctum were described. The pooled estimate through SMD suggested that herbal medicines were clinically and statistically superior to 0.12% CHX for parameters assessed at 15th and 30th day ($p<0.05$). There was no asymmetry or heterogeneity in the funnel plot, which suggests that publication bias is not present.

Conclusion: It was observed that herbs showed gradual improvement and are equally effective as CHX and could be used as an excellent adjunct to CHX in treating moderate to advanced periodontitis due to its minimal side effects.

***Keywords*:** Chlorhexidine, Gingivitis, Herbs, Plant extracts, Periodontitis, Systematic review

1.INTRODUCTION

Oral health is considered the mirror to systemic health and mouth is a gateway to person's overall health.[1] Periodontal disease (PD) is a health problem that causes tooth loss.[2] Additionally, it is among the most common oral diseases worldwide.[3] It is commonly known that microbial plaque is the primary risk factor of PD. Therefore, most important principle to control or eliminate it should be to either prevent it or treat it. Plaque control with mechanical devices such as toothbrushes or dental floss is insufficient and not without limitations.[4,5]

For this reason, importance is given to prevention as well as treatment with mouthwashes.[6,7]

Chlorhexidine has come to be a "standard" mouthwash due to its potent antibacterial properties, effectiveness and compatibility.[8] However, prolonged use of chlorhexidine might result in silicate and resin treatment, tooth, tongue, and gum stains, as well as dry mouth, ulcers, and taste alterations. It may also cause situations. Therefore, it cannot be used for daily protection.[9,10]

In recent times people are contemplating medicinal plants as an alternative.

Interestingly, in the field of periodontology, multiple herbs have been successfully utilized to reduce tooth plaque and eradicate oral diseases.[11,12] Patients are worried about their oral cleanliness and health in the modern period in which dentists operate.[13] As a result, there is mounting proof that oral and physical health are related.

Herbs and its "natural" components have long been used to safely and effectively restore health in a minimum hazardous way.[14–16] Herbal plants are both supportive and protective in their own way.[17] Many plants that are medicinal and extracts from them are frequently used to treat diseases and protect health.[18,19]

The main benefit of using these therapeutic plants is that there haven't been any documented negative effects thus far. Furthermore, alcohol and/or sugar, two prevalent constituents in the majority of commercially accessible products, are absent from all herbal products.[20]

Based on available data, no research has offered a thorough, quantitative, and comparative review of how herbal remedies and chlorhexidine affect periodontal diseases. Therefore, we conducted a systematic review and revised our study for pertinent studies in order to assess the efficacy of herbal treatments for periodontitis in contrast to 0.12% chlorhexidine.

2. MATERIAL AND METHODS

The PRISMA statement [21] was followed in this review, which was also filed under the Prospective Registration of Systematic Reviews (PROSPERO) number CRD42024506918.

Study design

The research question “Is there any difference in the efficacy of herbal medicines compared to 0.12% chlorhexidine on periodontitis in terms of reduction in Plaque Index (PI), Gingival Index (GI), reduction in Clinical Attachment Level (CAL), periodontal pocket depth (PPD) and colony forming unit (CFU)?” was put out in the Participants (P), Intervention (I), Comparison and Outcome (O) framework.

Eligibility Criteria

a) Inclusion Criteria:

1) Articles have been selected with no time and language restrictions

2) Studies published till January 2025 and having pertinent information about the effect herbal medicines compared to 0.12% chlorhexidine on periodontitis

3) Research presenting the results with regard to PI, GI, CAL, PPD, CFU

4) Research that displays data as frequency, mean, and standard deviation

5) Comparative studies and randomised controlled trials (RCTs) were included

6) The open access journal articles were selected

b) Exclusion Criteria:

1) Any research done prior to year 2000

2) Articles composed in non-English languages

3) Letters to the editor, editorials, abstracts, reviews, and animal studies were not included.

4) Articles from journals that are not open access

5) Articles that fail to provide the study's mean and standard deviation

Screening Process

Search and screening were done by two authors. The process of choosing of articles was divided into two phases. Two reviewers looked over the titles and abstracts of every article in first round. Articles that didn’t fit into the inclusion were removed. Phase-two, involved independent screening and review of few full papers by the same reviewers. Discussions were held to settle by any disputes. A third reviewer was bought in to make the ultimate decision when two reviewers could not agree upon something. All three authors came to agreement on choice in the end. When more information was needed, the studies corresponding authors were contacted by email.

Screening Process

Two authors conducted the investigation and assessment. There were two stages to the article selection process. In the first stage, two reviewers examined each article's abstract and title. Articles that were deemed unfit for inclusion were eliminated. A limited number of complete submissions were individually reviewed and assessed by identical reviewers in phase two. Any disagreements were resolved through discussions. When two reviewers couldn't agree on anything, the ultimate choice was made by a third reviewer. In the end, each of the three authors agreed with the choice. When more information was needed, emails were sent to the papers respective corresponding authors.

Search Strategy

To discover English-language literature, an electronic

search was conducted until January 2024 utilizing EBSCOhost, Google Scholar, and PubMed databases.

Medical Subject Heading (MeSH) terms and relevant keywords were chosen, and they were merged with Boolean operators such as AND: “herbal medicine” (MeSH term) AND “chlorhexidine” (MeSH term); “gingivitis” (MeSH term) AND “periodontitis” (MeSH term); “plaque index” (MeSH term) AND “gingival index” (MeSH term) AND “clinical attachment level” (MeSH term); “pocket depth” (MeSH term) AND “bleeding index” (MeSH term) AND “microbiological analysis” AND “plaque control” AND “randomized controlled trials” (MeSH term); AND “comparative study”.

Data extraction

Final analysis of each included study comprised the following headings: author(s), study nation, study year, sample size, study design, herbal medications utilized, parameters analyzed, and conclusion.

Assessment of methodological quality

The Cochrane Collaboration Risk of Bias (ROB)-2 tool[22] was used to assess the methodological quality of the included clinical trials or randomized controlled trials (RCT) across its different areas in Review Manager (RevMan) 5.3 software.

Statistical analysis

All statistical analyses were carried out using RevMan 5.3 (Cochrane Collaboration, Software Update, Oxford, UK), with the standardized mean difference (SMD) serving as the summary metric.[23] Less than 0.05 was chosen as the significance level.

Assessment of heterogeneity

Any discrepancies in the estimations of the treatment effects of the various studies were evaluated for significance using the Cochran's test for heterogeneity.[24] If P was less than 0.01, it was decided that heterogeneity was statistically significant.

Investigation of publication bias To evaluate publication bias, Begg's funnel plot was employed. Effect size vs standard error was plotted in a funnel. The asymmetry of the funnel plot may indicate publishing bias.[25]

3. RESULTS

Study Selection

After duplicates were eliminated, 121 studies were eliminated from the reference list of included research. After that, articles with full text had been evaluated for eligibility, and those that didn't fit the requirements were removed. Fourteen studies fulfilled the qualifying criteria and were incorporated into the qualitative synthesis, whereas fourteen studies were incorporated into the meta-analysis, as shown in **Figure 1**.

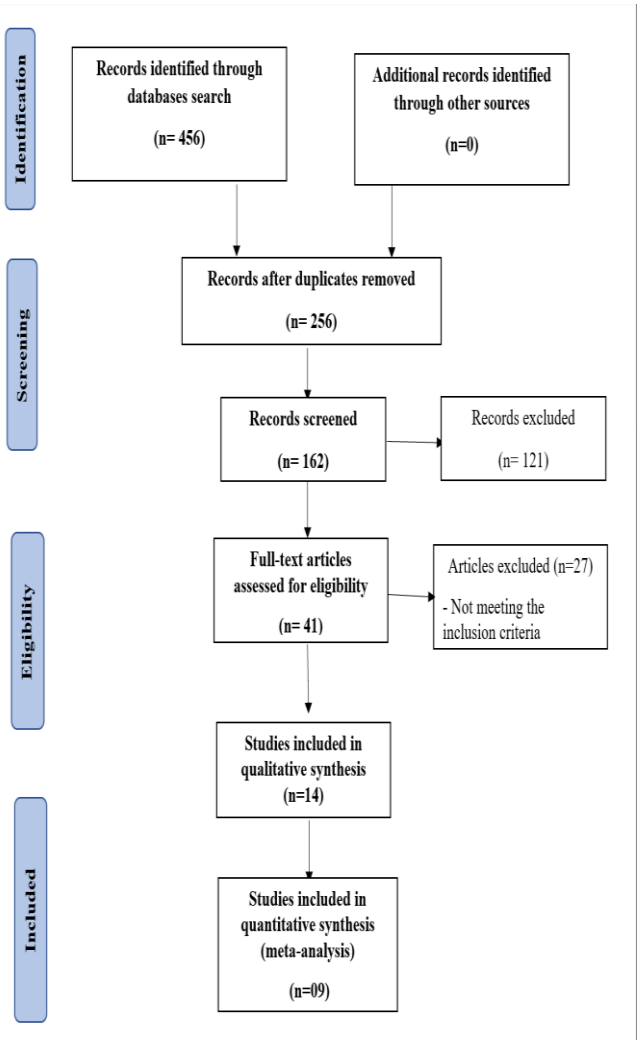


Figure 1. PRISMA Flow Diagram

Study Characteristics

Table 1 displays the results of fourteen research[6, 25–38]that assessed the effectiveness of 0.12% chlorhexidine and a variety of herbal remedies on a total of 1610 teeth. Clinical trial research designs were used in all of the included investigations. Twelve of the included studies[6, 25–29,33,35–38] were carried out in India, one in Malayasia[30], and one in Iran[32]. A variety of herbs has been described which included Punica granatum, Herboral (neem, tulsi, pudina, clove oil, ajwain, triphala), Morus alba, Natural curcumin, Salvadora persica, Khadiradi vati, Dashansanskar churana, Neem and Apamarga, Ferula assafoetida, Aloe vera, tea tree oil, Guava leaf extract, Ocimum sanctum. Various periodontal parameters like GI, PI, CAL, PD, CFU were evaluated between these herbs and 0.12% CHX being followed up for 15th and 30th day. From the results of studies, it was observed that herbs/herbal medicine had better plaque control, showed gradual improvement in controlling gingivitis and are as effective as CHX. The patients accept it biologically, and because it has fewer adverse effects than CHX, it may be a great alternative adjunct for treating intermediate to advanced periodontitis.

Table 1. Showing descriptive study details of included studies

Author, years of study	Country	Study design	Sample size	Herbal medicine used	Parameters evaluated	Follow up duration
Ahuja et al. 2011[25]	India	RCT	20	Punica granatum	GI, PI	15th
Malhotra et al. 2011[6]	India	RCT	50	Herboral (neem, tulsi, pudina, clove oil, ajwain, triphala)	PI	15th day
Gupta et al. 2014[27]	India	RCT	180	Morus alba	PI, GI	15th, 30th day
Anitha et al. 2015[28]	India	RCT	30	Natural curcumin	CAL, PPD	30 day
Prasad et al. 2015[29]	India	RCT	150	Salvadora persica	GI, PI	1st and 5th day
Abdulbaqi et al. 2016[30]	Malaysia	RCT	14	Salvadora persica L	PI	24hrs and 6th day
Mittal et al. 2018[31]	India	RCT	480	Khadiradi vati, Dashansanskar churana, Neem and Apamarga	CPITN	7th day, 30th day and 90th day
Hashemi et al. 2019[32]	Iran	RCT	126	Ferula assa-foetida	PI, GI`	15th day
Kamath et al. 2019[33]	India	RCT	120	Aloe vera, tea tree oil	GI, PI, CFU	30th day
Nayak et al. 2019[29]	India	RCT	60	Guava leaf extract	GI, PI, CFU	30th day
Penmetsa et al. 2019[35]	India	RCT	60	Ocimum sanctum, Aloe vera	GI, PI, BI	15th, 30th day
Saima et al. 2019[36]	India	RCT	90	Herbal rinse	GI, PI	15th day
Siddharth et al. 2020[37]	India	RCT	50	2% curcumin	BI, GI, PI, CAL, PPD	30 day
Gunjal et al. 2024[38]	India	RCT	180	Morus alba	GI, PI, PPD	15th and 30th day

BI: bleeding index; CAL: clinical attachment level; CFU: colony forming unit; CHX: chlorhexidine; CPITN: community periodontal index treatment needs; GI: gingival index; PI: plaque index; PPD: periodontal pocket depth; RCT: randomized controlled trial

Assessment of methodological Quality

Random sequence generation and participant and staff blinding had the highest ROB. The ROB of each included study ranged from moderate to low. The lowest ROB was assigned to the domains of allocation concealment, blinding of outcome assessment, insufficient outcome data, selective reporting, and other bias. **Figures 2 and 3** show ROB for individual studies as well as for other domains.

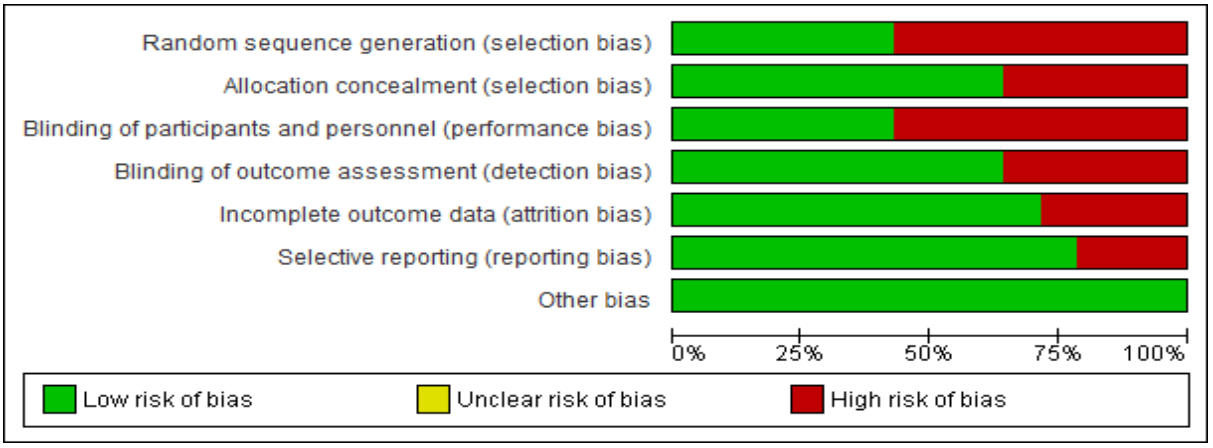


Figure 2. showing ROB graph: presented as percentages across all included studies.

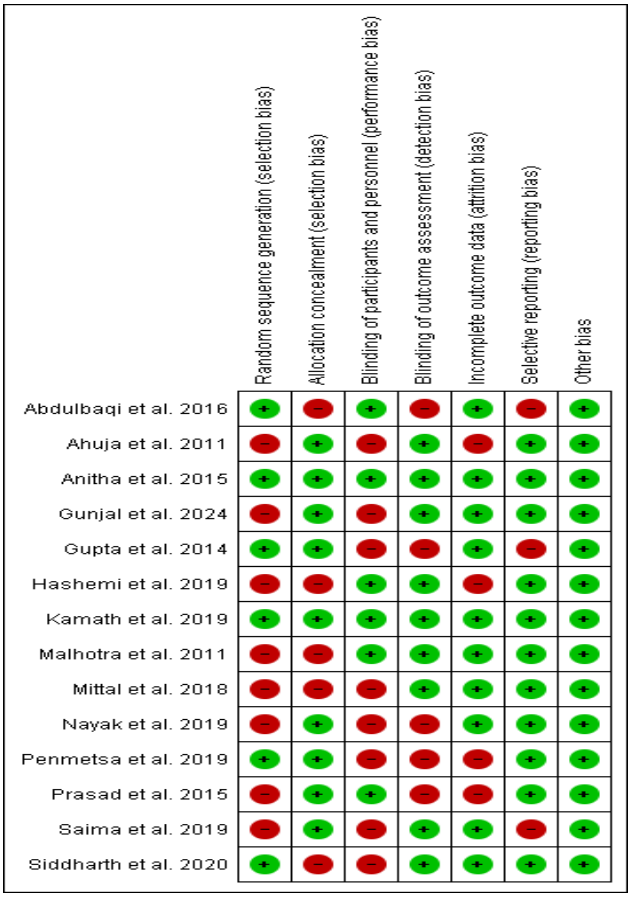


Figure 3. ROB summary: for each included study

Synthesis of results

A meta-analysis was conducted to evaluate the impact of herbs/herbal medicine compared to CHX on various periodontal parameters as shown in figures **4-11.** The parameters were assessed at 15th and at 30th day respectively.

Parameters evaluated at 15th day

Plaque index

Six studies [6, 25–27,32,35,36] with data on 268 patients examined (n=134) patients by CHX 0.12% group and (n=134) patients by herbal group to determine which was more successful in lowering the Plaque Index.

As observed in **Figure 4**, the SMD is 0.83 (-3.69 - 0.03) and the pooled estimates favours the herbal group, indicating that the herbal group has an average reduction in plaque index that is 0.83 times greater (p>0.05).

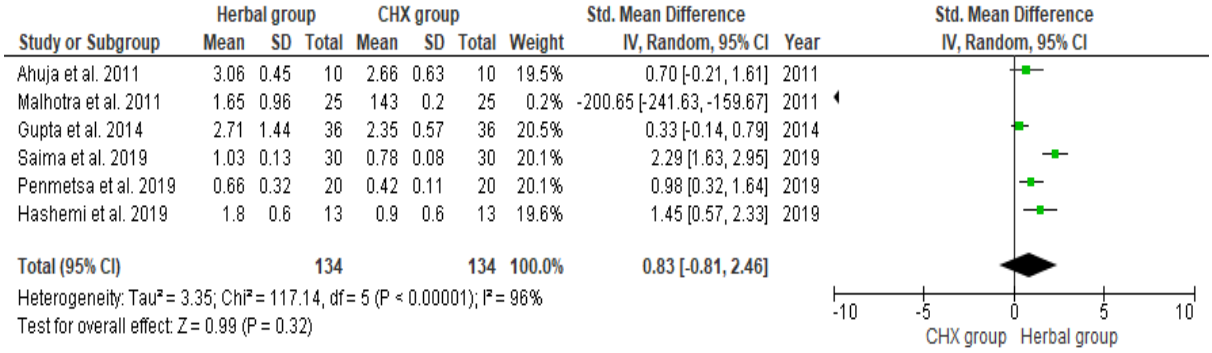


Figure 4. Plaque index between 0.12% CHX and herbal group at 15th day

Gingival index

Five studies had 218 patients in total[25,27,32,35,36] that examined (n=109) patients by CHX 0.12% group and (n=109) patients by herbal group to determine which group was more effective in lowering gingival index. As shown in **Figure 5**, the SMD is 0.68 (0.22 – 1.14) and the pooled estimates favours herbal group signifying that overall greater gingival index reduction on an average is 0.68 times greater in herbal group (p<0.05).

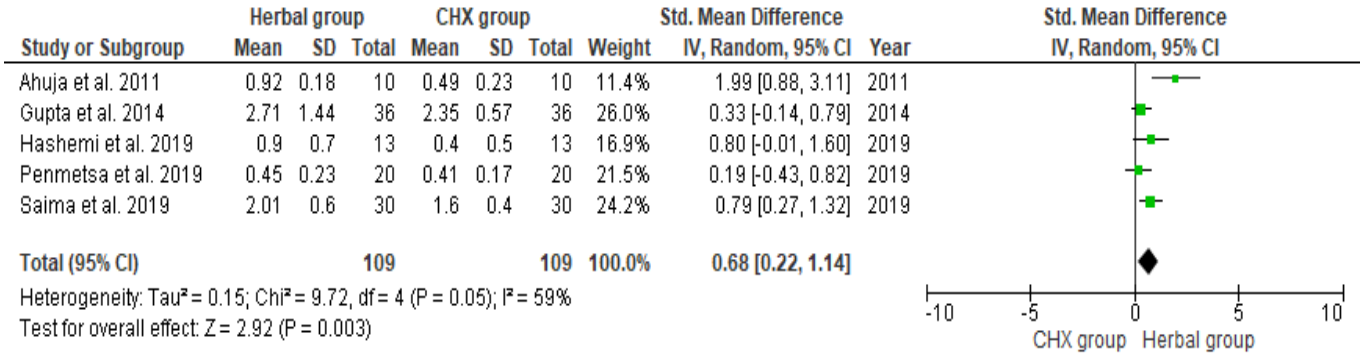


Figure 5. Gingival index between 0.12% CHX and herbal group at 15th day

No asymmetry was visible in the funnel plot, as shown in **Figure 6,** indicating that publication bias did not exist.

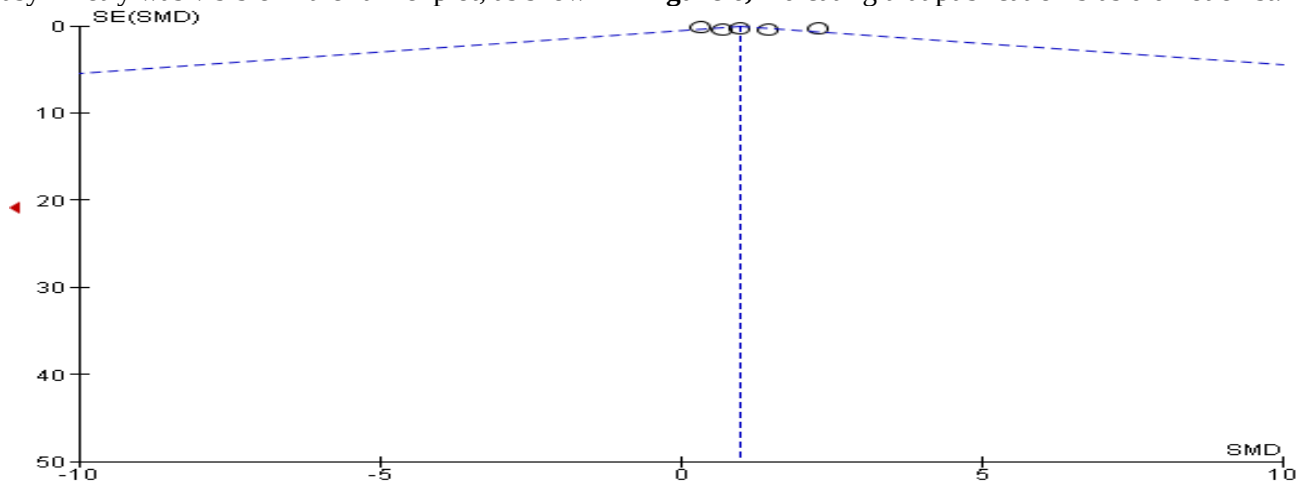


Figure 6. Begg's Funnel plot with 95% confidence intervals showing absence of publication bias

Parameters evaluated at 30th day

Microbial analysis (CFU)

Two studies [33,34] with data on 120 patients tested both the herbal group (n = 60) and the CHX 0.12% group (n = 60) to see which was more effective in reducing colony forming units.

Figure 7 illustrates that the herbal group has a greater overall decrease in colony forming units, with an SMD of 1.87 (01.65 – 5.38), and the pooled estimates favor the herbal group (p>0.05).

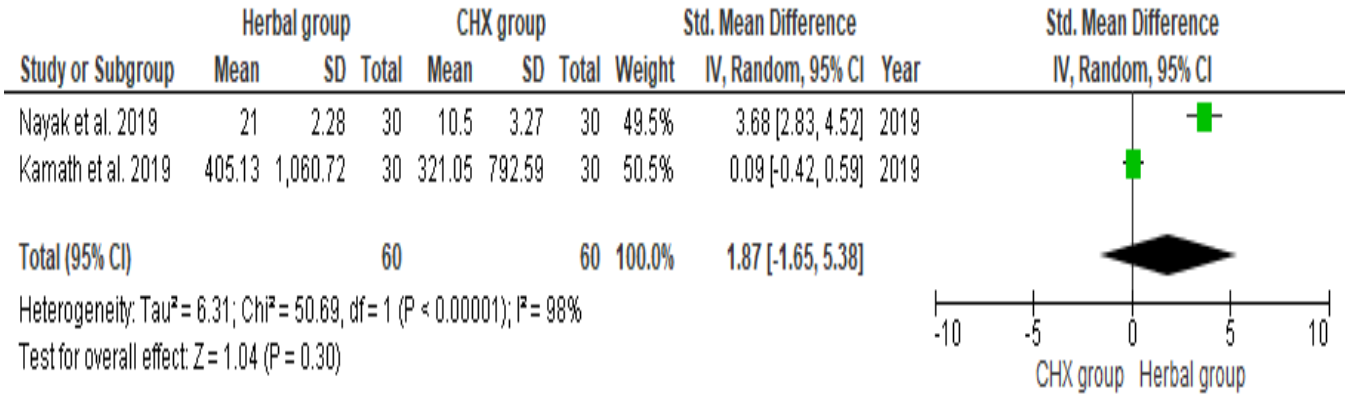


Figure 7. Microbial analysis between 0.12% CHX and herbal group at 30th day

Periodontal Pocket Depth (PPD)

In two studies [28,37] with data on 110 patients,(n= 55) patients were assessed by the CHX 0.12% group and (n=55) patients by the herbal group to determine which was more effective in reducing pocket depth.

As shown in **Figure 8**, the herbal group has a greater overall reduction in pocket depth on average by 1.37 times (p>0.05), with the SMD being -1.37 (-3.20 – 0.45), and the pooled estimates favoring the herbal group.

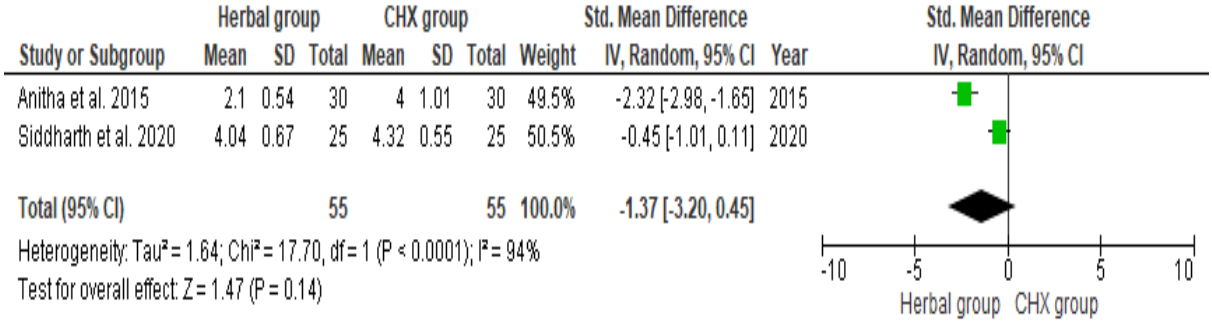


Figure 8. pocket depth between 0.12% CHX and herbal group at 30th day

Clinical Attachment Level (CAL)

Two studies[28,37] including information on 110 individuals, (n= 55) of whom were assessed by CHX 0.12% group and (*n*=55) patients by herbal group in order to evaluate for the better efficacy in lowering the clinical attachment level.

Figure 9 illustrates that the SMD is -1.39 (-3.19 – 0.42) and the pooled estimates favor the herbal group, indicating that the herbal group has an average 1.39-fold larger drop in attachment level (p>0.05).

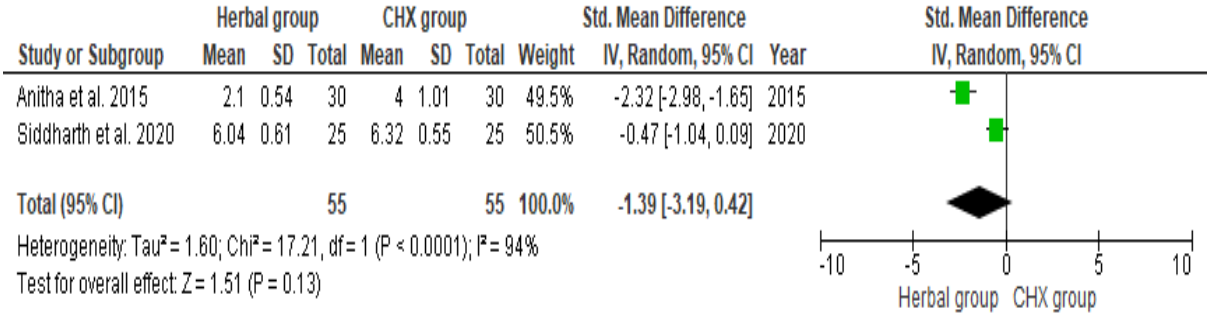


Figure 9. Clinical attachment level between 0.12% CHX and herbal group at 30th day

Gingival Index (GI)

Four studies [27,33–35] including information on 208 individuals, of whom (n=104) were assessed by the CHX 0.12% group and (n=104) by the herbal group to determine which group was more effective in lowering gingival index.

As illustrated in **Figure 10**, the SMD is 2.29 (-0.05 – 4.64), and the pooled estimates favor the herbal group, indicating that the herbal group has an average reduction in gingival index that is 2.29 times greater (p>0.05).

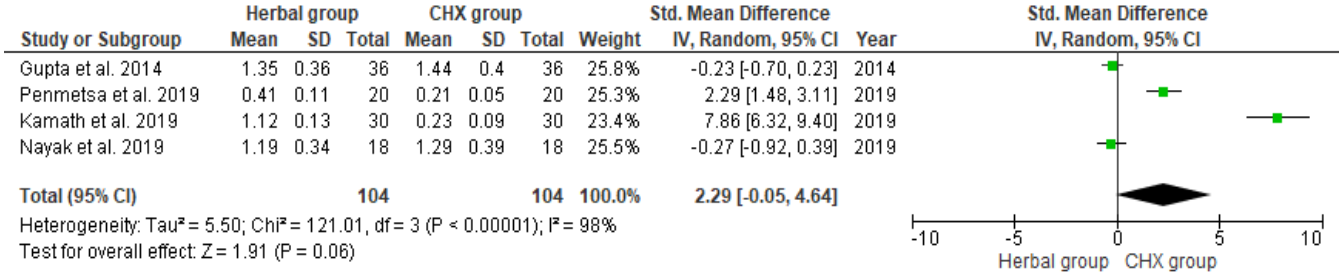


Figure 10. Gingival Index between 0.12% CHX and herbal group at 30th day

Plaque Index PI

Four studies [27,33–35] comprising 208 patient data, of which (n=104) patients were assessed by the CHX 0.12% group and (n=104) patients by the herbal group in order to determine whether group was more effective in lowering it.

Figure 11 illustrates that the SMD is 2.46 (0.55 – 4.37) and the pooled estimates favor the herbal group, indicating that the herbal group has an overall greater reduction in plaque index on average, which is 2.46 times greater ($p<0.05$).

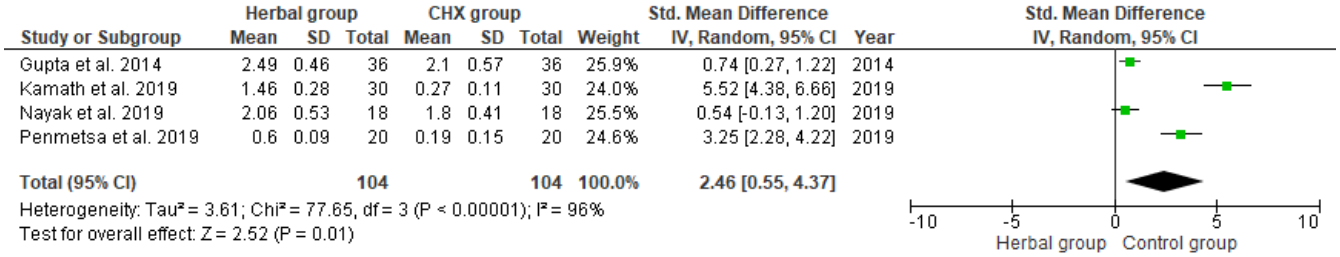


Figure 11. Plaque Index between 0.12% CHX and herbal group at 30th day

There was noticeable asymmetry in the funnel plot, which suggested the existence of publishing bias in **Figure 12.**

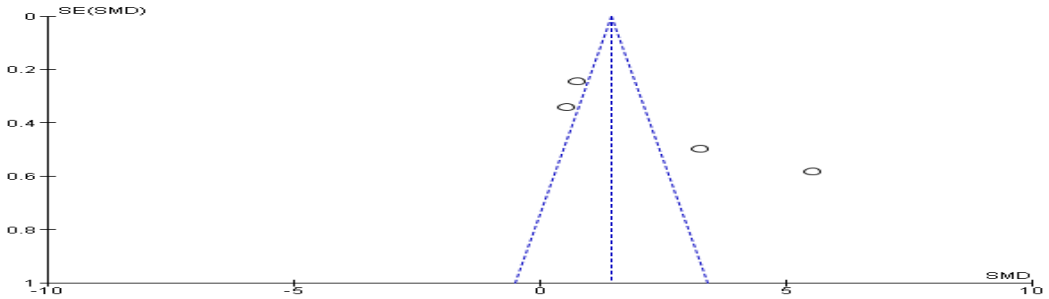


Figure 12. Begg's Funnel plot with 95% confidence intervals presence of publication bias

DISCUSSION

Dos santos et al.[39] performed a systematic review of RCTs with the aim to assess the effect of medicinal plants on dental biofilms. 47 studies were involved in review with herbs like Melaleuca alternifolia, Azadirachta indica and Ricinus communis oil compared against CHX for evaluating parameters like GI, PI and microbial count analysis. Based on the study's findings, it was determined that these herbs work equally as effectively as CHX. and could be a safer treatment for treating periodontal conditions.

The impact of curcumin as an alternate supplement to scaling and root planning was quantitatively analyzed by Zhang et al.[40] in comparison to CHX. Nine RCTs were found when databases were examined till February 2021. PI, GI, CAL, and PPD were evaluated at 3, 4, 6, and 12 weeks. It was observed that no significant difference was seen on reduction in attachment level while effect on GI and PI were similar to that of CHX.

Chatzopoulos et al.[41] observed a systematic review on effectiveness of herbal medicines in patients with periodontitis compared to conventional mode of treatment (scaling and root planning or supragingival debridement). Databases were searched or randomized and non-randomized trial till March 2022. From the finding of the study, it was concluded that herbal products showed better superior and effective clinical periodontal outcomes compared to conventional treatment.

Pasupuleti et al.[42] executed a systematic review on efficacy of herbal medicines within periodontal therapy. Databases were searched till June 2022. 14 studies fulfilled their eligibility criteria, of which eight were original studies and six were review articles. Glycyrrhiza glabra, ficus religiosa, plantago major,

medicago sativa, trifolium pratense, mangifera indica, pongamia pinnata, husk of Cocos nucifera, the root of g. glabra, curcuma longa, leaves of psidium guajava, azadirachta indica, fruits of citrus medica, punica granatum, ocimum moringa oleifera extract, and pomegranate peel extracts were among the various herbs that were included in the review. It was discovered that these herbs' alkaline properties, which have antibacterial properties, stop plaque and calculus from forming. Additionally, because of their anti-inflammatory and antioxidant qualities, these herbs may be used as a supplement to regular oral prophylactics.

This is the novel systematic review and meta-analysis that assesses impact of various herbs and herbal medicines on periodontal diseases and metrics, compared to 0.12% CHX. Databases were searched till January 2024 yielding 14 RCTs assessing the effect of herbal medicines compared to 0.12% CHX on parameters like GI, PI, CAL, PD, CFU at 15th and 30th day. A variety of herbs has been described which included Punica granatum, Organic curcumin, Morus alba, Herboral (neem, pudina, tulsi, clove oil, ajwain, and triphala), Salvadora persica, Khadiradi vati, Dashansanskar churana, Neem and Apamarga, Ferula assa-foetida, Aloe vera, tea tree oil, Guava leaf extract, Ocimum sanctum.

Reviews are widely regarded as the finest available evidence. But the level of quality the incorporated research determines how strong the evidence is. The current evaluation comprised enough studies with an identified risk of bias and a brief observation time.

Therefore, the evidence currently available is enough to provide therapeutic recommendations in answer to the focus issue of the current systematic review.

5. CONCLUSION

It was observed and demonstrated through the meta-analysis that herbal extracts had better plaque control, showed gradual improvement in controlling gingivitis and are as effective as CHX. Patients accept it biologically, and because it has fewer adverse effects than CHX, it may be a great alternative adjunct for treating moderate to advanced periodontitis.

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 ARTICALE

EVALUATION OF HYOID BONE POSITION AND ORIENTATION TO THE CRANIAL BASE, MANDIBLE, AND THIRD CERVICAL VERTEBRA IN DIFFERENT ANTEROPOSTERIOR SKELETAL MALOCCLUSION: AN OBSERVATIONAL STUDY

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ABSTRACT

Background: The aim of this study was to evaluate the hyoid bone (HB) position and it's orientation to cranial base, mandible, and third cervical vertebra in different anteroposterior skeletal malocclusion.

Materials and Methods: This retrospective study included 90 lateral cephalometric radiographs of Yemeni patients distributed into 3 groups with different skeletal classes of malocclusion aged between 18-30 years. All radiographs were traced manually by a researcher. The recorded data was collected, tabulated and analyzed by statistical program SPSS.

Results: The S-N (Sella-Nasion) distance, was low in Class III (69.5 mm) and the Hy-D distance was slightly higher in Class III. For, angular measurements, the NSAr (Nasion-Sella-Articulare angle) was high in Class III (128.2°) and SNA (Sella-Nasion-A point angle), was about 78.6 in Class III. A statistically significant difference was found in the S-N length ($p = 0.03$), indicating a longer cranial base in males. In females, the S-N distance correlated strongly with C3-HY ($r = 0.73$), while in males, C-Xi had a high correlation with Hy-D ($r = 0.82$). Angular measurements also showed strong correlations, such as SNA being negatively correlated with ArGoMe (-0.91) in females and NSAr negatively correlated with SNA (-0.77) in males, indicating cranial base influence on mandibular position.

Conclusions: The positioning of HB in different classes of skeletal malocclusion is a critical consideration in orthodontic therapy. The findings indicate significant variations in HB position and its orientation to other structures, emphasizing the need for clinicians to account for these differences during treatment planning.

Keywords: Hyoid Bone, skeletal malocclusion, malocclusion, prognathic maxilla, retrognathic mandible

INTRODUCTION

In orthodontics, understanding skeletal problems linked to malocclusion is essential. Numerous investigations utilising cephalometric radiographs have confirmed a correlation between the anatomy of the cranial base and the interaction of the jaw base. The cranial basis delineates both the skull and the facial bones.[1] The nasomaxillary complex is associated with the anterior cranial base, while the mandible is associated with the posterior cranial base.[2] The shape and structure of the anterior cranial base therefore have a significant impact on the position of the maxilla. The sagittal misalignment of the jaws is intricately linked to alterations in the cranial base.[3] The length and orientation of condylar growth, along with the displacement of the mandibular body due to the sutural growth of the cranial base, influence mandibular posture.[4]

The angle formed between the sella-nasion-articular (NSAr) and sella-nasion (NSBa) points is frequently utilised to radiographically assess the cranial base angle, also known as the saddle angle.[5] At birth, this angle measures 142 degrees, but by the age of five, it has gradually diminished to 130 degrees. This angle is rather stable between the ages of 5 and 15. The alignment of the jaws is profoundly affected by the growth and development of the cranium.[6] A predisposition for class II malocclusion may arise if the angle between the fossae increases.[7]

In the last two decades, there has been significant research in the hyoid bone (HB) and its correlation with facial

patterns.[8] Certain physiological activities, including phonation, deglutition, and respiration, rely on HB. It is an essential element of the diagnostic instrument utilised to assess the prognosis of orthodontic treatment and evaluate the probability of recurrence post-treatment.[9] Amayeri et al. found that large distance alteration was noticed between HB and the third cervical vertebra in Class III (C3M) cases than in Class I(C1M) and Class II (C2M) cases.[10] As a result, comprehending the relationship between HB and adjacent structures is crucial for elucidating the aetiology of malocclusion.

Investigators have discovered that changes in mandibular positioning correlate with adjustments in HB, which responds to variations in the anterior-posterior alignment of the head.[8,11] Recent decades have witnessed significant focus on investigating the impact of HB and its association with face traits. Previous research demonstrates a substantial correlation between the positional changes of HB and malocclusion. Therefore, the present study assessed and evaluated HB position and orientation to cranial base, mandible, and third cervical vertebra in different skeletal malocclusion.

2. MATERIAL AND METHODS

The present retrospective cross-sectional study was conducted carried out at Orthodontic Department, faculty of dentistry Sana'a University among a group of Yemeni adult population to analysis the relationship between HB position and Orientation to cranial base, mandible and cervical vertebra with different skeletal classes of malocclusion after obtaining an ethics approval from ethics from Institutional of the Medical Ethics Committee at the Faculty of Dentistry of Sana'a University with Ethical Approval Reference Number: OR: 06/ 09/ 2024.

Study Sample

A total of 90 subjects were classified into three groups and each containing 30 patients. The selected patterns were Class I (normal occlusion), Class II (retrognathism with a small mandible), and Class III (prognathism with a forward-positioned mandible). All radiographs were traced manually by a researcher. All subjects included in this study had been prescribed digital lateral Cephalometric image as a part of their diagnostics. The digital lateral cephalometric images were included if they were good quality radiographs permitting the identification of landmarks and taken from the same Machine. They were excluded if they were images with orthodontic treatment, severe long or short face, congenital anomalies like cleft lip and palate or had missing, supernumerary, prosthetic or implant placement. Variables like age and gender, and skeletal malocclusion were recorded.

Study Procedure

Digital lateral cephalograms were used to assess HB position. The radiographs were taken from Cephalometric machine Ex3Di-Vatech machine from Al-Weed X-ray center. The midsagittal plane was perpendicular to the floor and parallel to the film and the teeth are in maximum intercuspation in natural head position which means the standardized and reproducible position of the head in upright posture, with the eyes focused on a point in the distance at eye level, that indicates the visual axis is horizontal. The cephalometric radiograph was obtained at 85 KV and 10 mA per second. For stabilization of the head, the cephalometric machine's ear-rods put in the external auditory meatus and forehead support put in the forehead. The radiograph was taken with the same criteria to have the high-quality detectors for this study.

Manual Tracing Procedure

After collecting of data, the researcher saved them as JPG files, then printing them through Laser printer. Tracing was done by a .0.5 mm HB lead pencil, Eraser, millimeter ruler, geometric protractor with half degree approximation for the recording of linear and angular measurements and 8"x 10"matte acetate papers of 0.003mm thickness. Matte acetate paper was placed over the hard copy of lateral cephalometric radiographs and calibration was done before printing. The radiographs were traced in daylight by the researcher. Tracing was done to 90 cases. Photos of traced x-rays are taken with Nikon Digital Camera D5100 that is made in Thailand. 10 cephalograms were selected randomly after month and were retraced and measured by the same researcher in order to check the reliability of the angular and linear measurements that were measured before by Mortazavi S et al, (2018).[12] The Manual tracings were done by the researcher to reduce the measurements variability. The five linear measurements (in mm) were analyzed in this study were Sella-Nasion (S-N), Capitulare-Xi (C-Xi), C3-Hy(C 3-Hy), Hyoidale-Pt. D (Hy-D), and AA'-PNS. The seven Angular Measurements (in degree) that is used in this study were Saddle angle (NSAr), Articulare angle (SArGo), Gonial angle (ArGoMe), SNA, SND, Hyoidale angle (C3 HyD), and Hyoid plane angle.



Figure 1: Lateral Cephalometric radiograph from the studied Sample Demonstrating proper head position and landmarks marked on tracing sheet for manual tracing

Statistical analysis

The data was collected and analyzed and coded before being input into the statistical software (SPSS) application. Data was analyzed using statistical software R version 4.4.0 and Microsoft Excel. Continuous variables given in Mean ± SD / Median form. Student's t test was used sexual dimorphism in linear and angular measurements of males and females. P-value less than or equal to 0.05 indicates statistical significance.

3. RESULTS

The dataset comprises measurements from 90 subjects. The participants were equally categorized into the three skeletal classes. Each class contained 30 participants, ensuring an even distribution. The gender distribution is evenly split, with 45 (50%) females and 45 (50%) males.

Table 1 presents the mean and standard deviation (SD) of various linear measurements. The S-N (Sella-Nasion) distance, was highest in C2M (71.7 mm) and lowest in C3M (69.5 mm), suggesting cranial base length variations among skeletal classes. The Hy-D (Hyoid-Mandible distance) was slightly higher in Class III, indicating a possible anterior shift of HB. The C3-HY (Hyoid-Third Cervical Vertebra distance) was lowest in C2M, which may imply a retracted hyoid position in retrognathic individuals.

Table 1. Comparison of Linear Measurements parameters in skeletal groups

Parameters	C1M	C2M	C3M
	Mean±SD	Mean±SD	Mean±SD
S-N	70.9±3.17	71.7±3.54	69.5±4.5
C-Xi	35.3±3.44	34.9±4.15	35.2±4.23
Hy-D	42.2±5.2	42.1±5.1	43.6±7.87
C3-HY	38.1±9.13	35.8±3.99	37.3±5.68
AA'-PNS	32.4±5.03	35.5±8.36	32.6±3.82

In **Table 2,** Angular measurements were compared among skeletal classes. The NSAr (Nasion-Sella-Articulare angle) was highest in C2M (129.4°), suggesting an increased cranial base flexion in these individuals. The SNA (Sella-Nasion-A point angle), an indicator of maxillary positioning, was highest in C2M, confirming a more pronounced maxilla. The SND (Sella-Nasion-D point angle) was highest in C3M, reflecting a more forward-positioned mandible.

Table 2. Comparison of Angular Measurement parameters in skeletal groups

Parameters	C1M	C2M	C3M
	Mean±SD	Mean±SD	Mean±SD
NSAr	125.9±5.81	129.4±5.97	128.2±10.69
SArGo	143.9±7.41	142.9±7.5	140±6.75
ArGoMe	127.4±6.48	126.2±6.81	131±10.75
SNA	79.8±3.21	81.8±4.54	78.6±4.49
SND	74.8±4.3	72.7±3.66	77.4±5.22
C3HyD	158.4±12.58	155.5±16.66	158.8±12.58
Hyoid Plane Angle	92.7±7.27	93±5.1	90.2±4.82

The Principal Component Analysis accounted for 45.56% of variance in linear measurements while PC2 adds another 23.78% and together they explain 69.34% of the variance **(Table 3)**. The analysis indicates that PC1 and PC2 stand out as the primary components for capturing dataset patterns. PC1 leads all components with the maximum eigenvalue of 2.28 which establishes its key importance whereas PC2 retains significance in data analysis with its eigenvalue of 1.19. The variables S-N, CXi, and C3-HY show the highest loadings on PC1 which demonstrates their primary impact on this principal component **(Figure 2)**. In angular measurements, the PCA analysis demonstrates

that PC1 stands out as the primary component by explaining 51.96% of the variance while PC2 accounts for 20.6% and PC3 accounts for 15.57%. The combination of these three components explains 88.13% of the variance which shows they provide an effective summary of the dataset. PC1 (3.64), PC2 (1.44), and PC3 (1.09) show their importance in explaining the dataset structure because their eigenvalues are above 1 whereas PC4 through PC7 have eigenvalues below 1 which makes their contribution to total variance minimal. PC1 demonstrates the most substantial representation from the variables SArGo and SNA because they show the highest factor loadings on this principal component. **(Figure 3).**

Table 3. Factor Analysis of Linear and Angular Measurements

Linear Measurements							
Variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**		
S-N	0.5062	0.0735	0.4922	-0.6801	0.1835		
CXi	0.5336	-0.2705	-0.2988	0.3314	0.6656		
C3-HY	0.5373	0.051	0.3865	0.5477	-0.5092		
HyD	0.3963	0.4478	-0.6911	-0.2443	-0.3243		
AA-PNS	-0.1153	0.8475	0.2038	0.2608	0.3985		
Eigen Value	2.28	1.19	0.71	0.49	0.33		
% explained	45.56	23.78	14.22	9.79	6.65		
Angular Measurements							
Variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**
NSAr	-0.438	-0.2225	-0.2723	0.4953	-0.0403	-0.1843	0.6353
SArGo	0.4654	-0.1946	0.077	-0.4626	0.3325	-0.1176	0.6334
ArGoMe	-0.2934	0.6588	-0.0957	-0.1878	0.1704	0.5589	0.3067
SNA	0.4448	-0.2622	0.0694	0.5104	0.0671	0.6792	0.0479
SND	0.3491	0.4473	-0.3421	0.3925	0.5129	-0.3577	-0.1264
C3HyD	-0.0223	0.288	0.8709	0.2978	0.0188	-0.2008	0.1694
Hyoid Plane Angle	-0.4304	-0.3562	0.1745	-0.046	0.7687	0.1015	-0.2326
Eigen Value	3.64	1.44	1.09	0.54	0.18	0.07	0.04
% explained	51.96	20.6	15.57	7.72	2.57	1.07	0.52

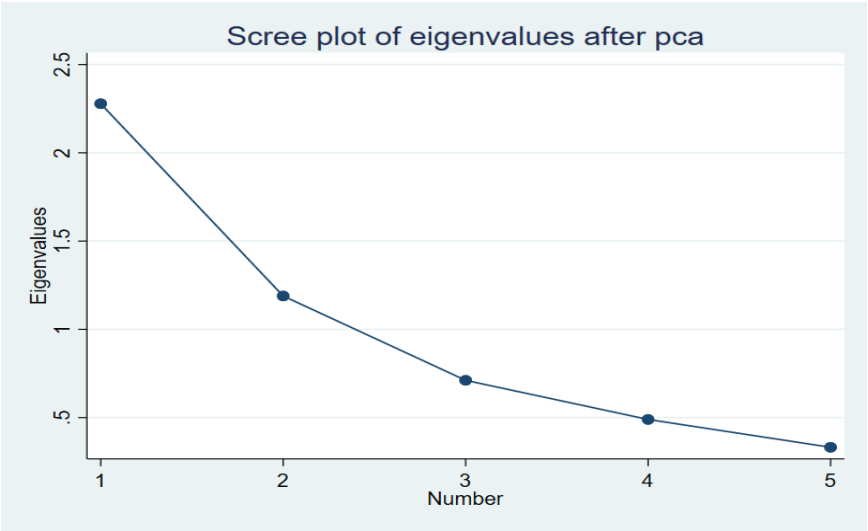


Figure 2. Scree Plot – Linear measurements

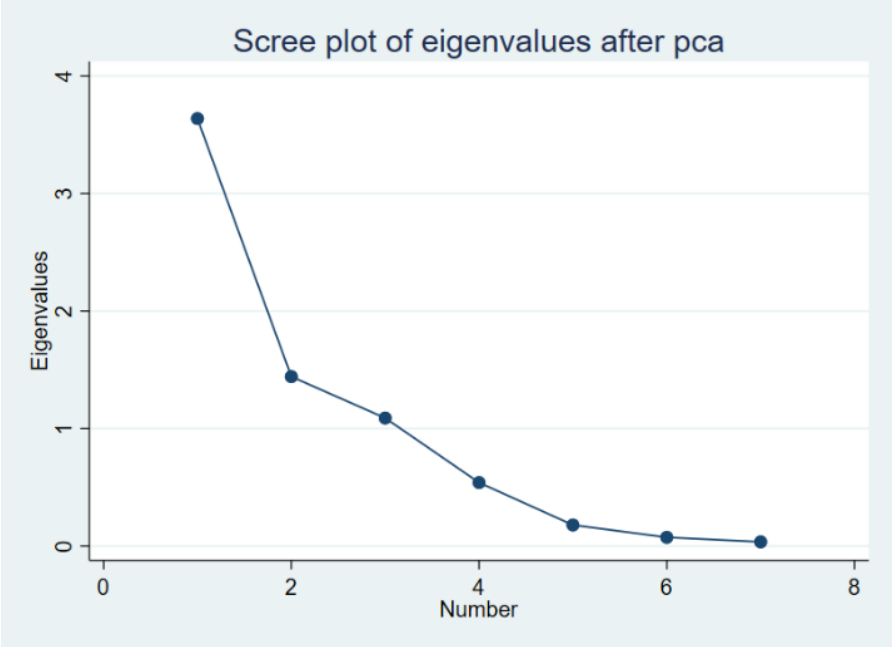


Figure 3.Scree plot – Angular Measurements

Table 4. Correlation matrix for linear measurements – female (95% CI)

	S-N	C-Xi	C3-HY	Hy-D	AA'-PNS
S-N	**1.00**				
C-Xi	**0.23**	**1.00**			
C3-HY	**0.73***	**0.67***	**1.00**		
Hy-D	**0.27**	**0.06**	**0.64***	**1.00**	
AA'-PNS	**-0.65***	**-0.73***	**-0.55***	**0.29**	**1.00**

***Statistically Significant**

Table 5. Correlation matrix for linear measurements – male (95% CI)

	S-N	C-Xi	C3-HY	Hy-D	AA'-PNS
S-N	**1.00**				
C-Xi	**0.44***	**1.00**			
C3-HY	**0.45***	**0.43***	**1.00**		
Hy-D	**0.47***	**0.82***	**0.21**	**1.00**	
AA'-PNS	**0.51***	**0.06**	**0.55***	**-0.06**	**1.00**

***Statistically Significant**

Table 6. Correlation matrix for linear measurements – total (95% CI)

	S-N	C-Xi	C3-HY	Hy-D	AA'-PNS
S-N	**1.00**				
C-Xi	**0.42***	**1.00**			
C3-HY	**0.55***	**0.53***	**1.00**		
Hy-D	**0.32***	**0.37***	**0.31***	**1.00**	
AA'-PNS	**-0.05**	**-0.33***	**-0.03**	**0.17**	**1.00**

***Statistically Significant**

Correlation matrix shows correlations between different linear and angular measurements for males, females, and the total sample. In females, the S-N distance correlated strongly with C3-HY (r = 0.73), while in males, C-Xi had a high correlation with Hy-D (r = 0.82). The total sample showed moderate correlations, suggesting that certain skeletal parameters are interrelated **(Tables 4–6)**.

Table 7. Correlation matrix for angular measurements – female

	NSAr	SArGo	ArGoMe	SNA	SND	C3HyD	Hyoid Plane Angle
NSAr	1.00						
SArGo	-0.52*	1.00					
ArGoMe	-0.39	-0.49	1.00				
SNA	0.69*	0.09	-0.91*	1.00			
SND	-0.60*	-0.26	0.59*	-0.55*	1.00		
C3HyD	0.31	-0.76*	0.16	0.17	0.56*	1.00	
Hyoid Plane Angle	0.69*	0.23	-0.92*	0.94*	-0.79*	-0.13	1.00

Table 8. Correlation matrix for angular measurements – male

	NSAr	SArGo	ArGoMe	SNA	SND	C3HyD	Hyoid Plane Angle
NSAr	1.00						
SArGo	-0.85*	1.00					
ArGoMe	0.65*	-0.89*	1.00				
SNA	-0.77*	0.86*	-0.79*	1.00			
SND	-0.39*	0.46*	-0.26	0.68*	1.00		
C3HyD	-0.43*	0.10	0.18	-0.07	-0.32	1.00	
Hyoid Plane Angle	0.81*	-0.85*	0.71*	-0.94*	-0.81*	0.10	1.00

Table 9. Correlation matrix for angular measurements – total (95% CI)

	NSAr	SArGo	ArGoMe	SNA	SND	C3HyD	Hyoid Plane Angle
NSAr	1.00						
SArGo	-0.81*	1.00					
ArGoMe	0.23	-0.63*	1.00				
SNA	-0.52*	0.70*	-0.75*	1.00			
SND	-0.50*	0.37*	0.05	0.47*	1.00		
C3HyD	-023	-0.11	0.17	-0.01	-0.10	1.00	
Hyoid Plane Angle	0.72*	-0.56*	0.13	-0.55*	-0.78*	0.05	1.00

Angular measurements also showed strong correlations, such as SNA being negatively correlated with ArGoMe (-0.91) in females and NSAr negatively correlated with SNA (-0.77) in males, indicating cranial base influence on mandibular position **(Tables 7–9)**.

DISCUSSION

The present study was undertaken to evaluate the position of HB and its orientation to cranial base, mandible and third cervical vertebra in different anteroposterior skeletal malocclusions. Out of 90 participants, for linear measurements, the S-N distance, was highest in C2M and lowest in C3M. The Hy-D was slightly higher in C3M, indicating a possible anterior shift of HB. The C3-HY was lowest in C2M, which may imply a retracted hyoid position in retrognathic individuals. When angular measurements were compared among skeletal classes, the NSAr was highest in C2M, suggesting an increased cranial base flexion in these individuals. The SNA was highest in C2M, confirming a more pronounced maxilla. For angular measurements, our study suggested an increased cranial base flexion in the included patients. Our study found no statistically significant differences in linear and angular measurement across groups, indicating class alone not strongly influence these parameters. Thus, our null hypothesis that there is no agreement of presence of correlation between cranial bases, mandible and third cervical vertebra with HB position and orientation in different anteroposterior skeletal malocclusion in cephalometric x-rays was rejected.

A statistically significant difference was found in the S-N length, indicating a longer cranial base in males. The C3-HY distance was significantly higher in females, suggesting a gender-related difference in HB positioning. This suggests that the oropharyngeal dimension is smaller

in females than in males, as the HB is relatively positioned closer to the third cervical vertebra in females.

Our results are consistent with other studies. Breh R et al, 2017 that found anterior cranial base was shortest in skeletal C3M and longest in skeletal C2M.[13] Bhullar et al., in a similar 2020 study observed that the anteroposterior position of HB does not change with different growth patterns in skeletal C1M subjects.[14] Soheilifar et al., studied 50 cephalographs and observed that there is no statistically significant difference between HB position in skeletal C1M and skeletal C2M patients. Thus, as HB position is similar in skeletal C1M and C2M patients, the skeletal pattern must not be considered as the only determinant of the position of HB.[15] This is consistent with our findings on non-parametric comparisons of linear and angular measurements across skeletal classes which showed that no statistically significant differences were observed across groups, indicating that skeletal class alone does not strongly influence these parameters.

The anteroposterior position of the HB is the consequence of the activity of the suprahyoid muscles, which is associated with the clockwise and anticlockwise rotations of the mandible. HB is in an anterior and upward position during an anterior rotation. In the posterior, it is situated more retro-inferior. It is evident that the mandibular rotation has a significantly greater influence on the sagittal HB position than the classes.[16]

From our current study, PCA was performed to minimize data dimensions and identify which components best illustrate the data's variability. The analysis indicates that PC1 and PC2 stand out as the primary components for capturing dataset patterns. The result was that variables S-N, CXi, and C3-HY show the highest loadings on PC1 which demonstrates their primary impact on this principal component. The variables SArGo and SNA show the highest factor loadings on this principal component. PCA performed in similar studies has yielded agreeable findings.

Another study by Bedoya A et al., The anteroposterior position of the HB is the consequence of the activity of the suprahyoid muscles, which is associated with the clockwise and anticlockwise rotations of the mandible. HB is in an anterior and upward position during an anterior rotation. In the posterior, it is situated more retro-inferior. It is evident that the mandibular rotation has a significantly greater influence on the sagittal HB position than the classes.[17] Accordingly, Bibby and Preston discovered significant variability in the impact of HB position on head position, even with slight movements.[18] In this study, the variable that measures the position of HB was very stable and behaved independently; i.e. it was not related to any other variable.

Lastly, in our study correlation analysis shows that in females, the S-N distance correlated strongly with C3-HY, while in males, C-Xi had a high correlation with Hy-D. The total sample showed moderate correlations, suggesting that certain skeletal parameters are interrelated. Angular measurements also showed strong correlations, such as SNA being negatively correlated with ArGoMe in females and NSAr negatively correlated with SNA in males, indicating cranial base influence on mandibular position. Various studies have reported strong correlations with respect to HB positions and its orientations.

Deljo et al. (2012)[19] aimed to ascertain the position of HB in relation to the cranial base, mandible, and cervical region of the vertebrae. The position of HB is variable and contingent upon the maxillo-mandibular anterior-posterior relationships. The lengths of the HB and its greater horns vary in relation to sagittal malocclusion. The flat position of HB is highly correlated with the cranial base and maxillary bones. A positive correlation was identified concerning the cervical vertebra, while a dependence was established in relation to the steep mandibular plane. The flat position of HB is strongly correlated with the cranial base and maxillary bones. In patients from group II, the longest HB length was observed. A correlation between the length of the anterior cranial base and the length of the maxilla was also identified.[19] performed a correlation analysis and discovered that the hyoidale angle was also correlated with the angle. SNA exhibited a notable correlation in C1M normal subjects, whereas in C2M division 1 samples, it was statistically insignificant. An elevation in saddle angle (NSAr) and articulare angle (SArGo) results in a retrognathic mandibular configuration. The correlation of these angular measurements with the hyoidale angle revealed a statistically insignificant negative correlation in C1M normal subjects and C2M division 1 samples.[20] The gonial angle (ArGoMe) exhibited no significant correlation with the hyoidale angle in both classes. These findings reinforce the notion that skeletal malrelationship induces a shift in HB position, specifically indicating that sagittal malformation plays a highly significant role in the anteroposterior positioning of HB.

The low level correlation between saddle angle and hyoidale angle may be attributed to the fact that a large saddle angle is compensated for by a change in articulare angle and ramus length. In addition to influencing the anteroposterior positioning of the mandible, the articulare angle also influences the closing and opening of the bite. The articulare angle in C2M division 1 is increased by the distal mandibular position, which is compensated for by the deep overbite, resulting in a relatively minimal change in articulare angle.

A positive correlation was observed between the length of the mandible (measured from the points of GonMen) and the distance between the third cervical vertebra and HB, as described by Trenouth and Timms in 1999.[21] Ingervall et al. discovered a positive correlation (although not always statistically significant) between the anteroposterior distance between the retruded contact and intercuspal positions of the mandible and the vertical movement of HB between these positions.[22]

The mean value of C3-H for females was 3.57 ± 0.43, while for males it was 3.58 ± 0.43, as observed by Raghav et al. in their study. Consequently, they verified a statistically significant correlation between the Gonial angle of males and females.[23] Similarly, Jose et al. confirmed that a positive correlation was observed between the lower airway and the horizontal distance from the HB to the retrognathion in the C1M skeletal pattern with an average growth pattern. Nevertheless, no correlation was observed between the horizontal and vertical positions of HB in the C2M and C3M skeletal patterns and the normal growth pattern.[24]

The present study has a few limitations. The study population was restricted to a single institution. With larger cohorts, these findings can be broadened. Then all patients included in the study were from Yemen; hence, generalizing the results to individuals of other ethnicities would require further investigation. As the present study was a single-center study, the results may not be generalized to different ethnic groups. Next, severity of malocclusion in the participants was not classified, and patients with severe skeletal malocclusions may present with more severe compensatory characteristics, which may influence conclusions. Lastly, a limitation of current study was that the absence of functional assessment of HB during functions such as swallowing, speech and breathing which can be incorporated in future research.

5. CONCLUSION

The placement of HB in different types of skeletal malocclusion is a vital factor in orthodontic treatment. Our research reveals considerable discrepancies in the position and orientation of HB relative to other anatomical features among various skeletal classes, underscoring the necessity for clinicians to consider these variances in treatment planning. Moreover, considering the possible hazards of sleep apnoea and airway blockage, a thorough assessment of the patient's airway status is necessary before any surgical procedure to reduce the risk of problems.

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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ASSESSMENT OF PERCEPTION AND EXTENT OF ORAL HYGIENE PRACTICE AMONG COMILLA UNIVERSITY STUDENTS BY A DESCRIPTIVE CROSS-SECTIONAL STUDY

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ABSTRACT

Knowledge of oral health is a fundamental prerequisite for healthy behavior, allowing individuals to take measures to protect their overall health. To examine the perception and extent of oral hygiene behavior, this study was conducted among current 227 Students of Comilla University within age range of 18-27 years irrespective of gender, religion, and customs. IBM SPSS Statistics version 26.0, the Statistical Package for Social Sciences, was used for the analysis with a 95% CI, and the significance level was set at $p< 0.05$. Among the participants of this study, the majority were female (61.9%). Dental visits were significantly influenced by age ($p<0.05$) where the students whose ages were 18-22 years had 0.242 times [AOR=0.242; 95% CI: 0.070-0.826] less dental visit history than those of more than 25 years. Respondents having dental problems ($p<0.05$) including, dental cavities, dental caries, and toothache were more likely to visit dentists compared to the students who didn't have any dental problems (AOR=11.026; 95% CI: 3.747-32.440, AOR= 8.768; 95% CI: 1.501-51.191, and AOR=7.132; 95% CI: 2.021-25.163 respectively). More than half (55.9%) of the students had gum bleeding while brushing and are 2 times more likely to visit a dentist than those who didn't. Regarding oral hygiene practices, including type of toothbrush, brushing frequency, and using oral aids, there was no significant difference ($p>0.05$) in dental visits except for using toothpicks [AOR=0.283; 95% CI: 0.121-0.654]. Future studies are needed to determine whether this framework is supported by empirical data and leads to improvements in oral health.

Keywords: Oral health**;** Students**;** Dental caries**;** Dental visit**;** Oral hygiene

1. INTRODUCTION

The total lack of pain, discomfort, or illnesses pertaining to the mouth cavity and its associated structures, including the teeth, tongue, jaws, and oral soft and hard tissues, is known as oral health [1]. An individual's oral health has a direct impact on their oral functions and social relationships. It is also intimately related to their general health and quality of life [2,3]. Keeping the mouth and teeth clean is the practice of oral hygiene, which aims to prevent dental issues such as cavities, gingivitis, periodontal disorders, and bad breath [4].

Frequent dental checkups, flossing, and twice-daily tooth brushing are all examples of good oral hygiene habits that dramatically lower the incidence of oral diseases [5]. Furthermore,

following these guidelines has been connected to a higher socioeconomic level [5] and education level [6]. Maintaining good dental health starts with good oral cleanliness, which also prevents 80% of dental issues [7]. Keeping your mouth healthy starts with practicing basic dental hygiene [8]. Ensuring good dental health is crucial to one's overall health, happiness, and quality of life [9,10]. Throughout the world, oral illnesses represent a significant public health issue. In particular, people from lower socioeconomic classes are more likely to suffer from oral disorders [11]. Due to their high incidence and detrimental consequences on a person's quality of life, oral illnesses are a serious public health concern [12].

One of the most common oral disorders in children is dental caries, which is brought on by tooth plaque and is closely linked to inadequate oral hygiene habits [13-16]. Approximately 3.9 billion individuals worldwide are impacted by dental caries, periodontal disease, and oropharyngeal malignancies, which collectively contribute significantly to the disease burden [17,18]. In addition, bad dental health can lead to discomfort, pain, or dysfunction and has a detrimental effect on day-to-day activities, performance at work and in school, and quality of life [6,19].

Among all medical specialties, oral health receives very little attention globally. The prevalence of periodontitis alone is thought to be 45% in South Asians between the ages of 35 and 44 [18]. According to a prior study, dental caries was more common in children in Bangladesh than in adults between the ages of 18 and 35, and 45 across all socioeconomic levels [20].Previous studies show that due to cultural, religious, and belief-based conventions, a large number of people in South Asian nations lack basic knowledge about oral health and use antiquated or traditional oral hygiene practices [18], [21], [22]. Understanding oral health is a basic requirement for good behavior, which enables a person to take preventative action for their health. Several studies have demonstrated connections between improved oral hygiene and health-related behaviors and a greater understanding of oral health [23], [24].

While research on university students' oral health has gained importance worldwide, there is still a need for specific studies in regions like Cumilla to understand the particular difficulties and influencing factors that increase dental visits. The study aims to investigate the prevalence of dental visits among students of Comilla University. Moreover, this study determines the influential factors that may increase a student's dental visits. The study's findings will be useful for stakeholders in oral health care and policymakers in understanding Bangladesh's dental health prospects and developing appropriate policies for enhancing field-level services.

2. MATERIALS AND METHOD

Study design: A cross-sectional type of study was conducted to assess the level of knowledge about oral hygiene and the extent of oral hygiene practice among the students of Comilla University. In this three months long survey work, we gathered data from current students studying in different departments of Comilla University, Cumilla, Bangladesh. Students within age range of 18-27 years irrespective of gender, religion, and customs were included in this purposive sampling technique.

Human participants: Students were approached to share their knowledge if they would agree to. Participants were provided with clear and comprehensive information about the study, including potential risks and benefits, before giving their consent to participate. As required, this survey work was approved by the Biosafety, Biosecurity and Ethical Committee, Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka, Bangladesh with the **Ref No.: BBEC, JU/M 2024/08 (128).**

Statistical Analysis: A Microsoft Excel data sheet was used to record and evaluate the collected data, and IBM SPSS Statistics version 26.0, the Statistical Package for Social Sciences, was used for the analysis with a 95% confidence interval, and the significance level was set at $p< 0.05$.

Categorical variables are expressed as frequencies and percentages. Chi-square tests were used to identify significant connections between dependent and independent variables. The significant influencing factors of the dependent variables were used in the binary logistics regression model with the outcomes reported as odds ratios (OR) to assess the impact of selected factors.

2. RESULTS AND DISCUSSION

Table 1 shows the frequency and association of dependent and independent variables. A total of 227 students participated in this study where most of the respondent students were female (61.9%) and the rest 33.9% of respondent students were male. More than half (54%) of female respondents visited a dentist which is higher than male respondents (51.9%). The 18-22 year-old students (56.4%) were the maximum in number whereas only 10.1% of respondents were more than 25 years old. Around 43% of respondents who were 18-22 years aged visited a dentist and more than 65% of respondents who were greater than 25 years aged visited a dentist. Most of the students (62.6%) were students of the science faculty and more than half of the respondent students (55.5%) were from residence halls.

The non-science faculty respondents had a higher dental visit percentage (62.4%) and 58.4% of homestay respondent students visited a dentist. Only 4.8% of respondent students used hard-type toothbrushes and 52.5% used medium-type toothbrushes. The hard-type toothbrush users had a higher proportion (90.9%) of dental visits. More than half (52.4%) of the respondent brushes once a day and 62.6% of respondent students changed their toothbrushes in less than or equal to 4 months. The respondents who changed their brush more than 4 months interval had a higher percentage (56.5%) of dental visits. The maximum of students used mouthwash or toothpicks. The respondents who use mouthwash had a higher proportion (67.1%) of dental visits. Most of the respondents (60.8%) didn't forget to brush their teeth out of laziness whereas only 25.1% forgot to brush their teeth out of laziness. The respondents who forgot to brush out of laziness had a maximum of visits to a dentist.

The maximum of the students (34.4%) had tooth sensitivity dental problems, and only 4.4% had dental caries. The highest dental visits proportion of respondents had dental cavities, tooth sensitivity, and toothache dental problems. Over half 56% of respondents had gum bleeding while brushing and only 30% expressed fear in the dentist's chamber where 12.9% fear for toothache**.** Most of the respondents visited a dentist who had gum bleeding problems (59.8%) while brushing and didn't fear the dentist's chamber (57.9%). Only 19.8% of respondents believed that they had enough knowledge about taking care of their teeth and 55.1% had little knowledge. Most respondents (87.2%) didn't take care of their teeth before sleeping. Nearly two out of three students (69.2%) sometimes like to eat sweets. The respondents who always ate sweets had a high proportion (64.3%) of visiting a dentist. Only 24.5% of respondent students had some bad habits such as Biting nails (9.7%), Bruxism (5.7%), and Grinding (7.1%). Over half (52.4%) of respondent's students advise others to brush their teeth regularly and 18.5% had no advice to others. From the chi-square test, we found dental visits had a significant influence on age of the respondent, faculty, types of toothbrushes, the items the respondent used, dental problems, gum bleeding, fear of the dentist's chamber, like eating sweets, respondent advice for others to take care their teeth. We take those significant influencing variables in the binary logistic regression model.

Table 1. The frequency distribution and association between Socio-demographic factors and dental visiting history.

Variables	Overall N (%)	Dental Visit		P-value
		No (%)	Yes (%)	
Gender				
Female	150 (66.1%)	69 (46%)	81 (54%)	0.769
Male	77 (33.9%)	37 (48.1%)	40 (51.9%)	
Age of respondent				
18-22	128 (56.4%)	73 (57%)	55 (43%)	**<0.05**
23-25	76 (33.5%)	25 (32.9%)	51 (67.1%)	
>25	23 (10.1%)	8 (34.8%)	15 (65.2%)	
Faculty				
Non-science	85 (37.4%)	32 (37.6%)	53 (62.4%)	**<0.05**
Science	142 (62.6%)	74 (52.1%)	68 (47.9%)	
Residence Type				
Homestay	101 (44.5%)	42 (41.6%)	59 (58.4%)	0.167
Residence Hall	126 (55.5%)	64 (50.8%)	62 (49.2%)	
Which type of toothbrush do you use?				
Hard	11 (4.8%)	1 (9.1%)	10 (90.9%)	**<0.05**
Medium	120 (52.9%)	61 (50.8%)	59 (49.2%)	
Soft	96 (42.3%)	44 (45.8%)	52 (54.2%)	
Brush frequency				
Once	119 (52.4%)	61 (51.3%)	58 (48.7%)	0.148
Twice or More	108 (47.6%)	45 (41.7%)	63 (58.3%)	
How often do you change your toothbrush?				
≤ 4 months	142 (62.6%)	69 (48.6%)	73 (51.4%)	0.459
> 4 months	85 (37.4%)	37 (43.5%)	48 (56.5%)	
Which of the items mentioned below do you use?				
Dental floss	17 (7.5%)	7 (41.2%)	10 (58.8%)	**<0.05**
Miswak	57 (25.1%)	28 (49.1%)	29 (50.9%)	
Mouthwash	76 (33.5%)	25 (32.9%)	51 (67.1%)	
Toothpick	77 (33.9%)	46 (59.7%)	31 (40.3%)	

Do you forget to brush your teeth out of laziness?				
No	138 (60.8%)	63 (45.7%)	75 (54.3%)	0.900
Sometimes	32 (14.1%)	16 (50%)	16 (50%)	
Yes	57 (25.1%)	27 (47.4%)	30 (52.6%)	
Dental problem				
None of them	56 (24.7%)	42 (75%)	14 (25%)	
Dental Cavity	42 (18.5%)	11 (26.2%)	31 (73.8%)	**<0.05**
Dental Caries	10 (4.4%)	3 (30%)	7 (70%)	
Tooth Sensitivity	78 (34.4%)	38 (48.7%)	40 (51.3%)	
Toothache	41 (18.1%)	12 (29.3%)	29 (70.7%)	
Do you have gum bleeding while brushing?				
No	100 (41.1%)	55 (55%)	45 (45%)	**<0.05**
Yes	127 (55.9%)	51 (40.2%)	76 (59.8%)	
Do you fear the dentist's chamber?				
No	159 (70.0%)	67 (42.1%)	92(57.9%)	**<0.05**
Yes	68 (30.0%)	39 (57.4%)	29 (42.6%)	
Reason of fear				
Have no fear	159 (70.0%)	67 (42.1%)	92(57.9%)	0.050
Fear of dental instruments	17 (7.5%)	12 (70.6%)	5 (29.4%)	
Fear of injection	22 (9.7%)	14 (63.6%)	8 (36.4%)	
Fear of toothache	29 (12.8%)	13 (44.8%)	16 (53.3%)	
Do you believe you have enough knowledge about taking care of your teeth?				
A little	125 (55.1%)	60 (48%)	65 (52%)	0.874
No	57 (25.1%)	25 (43.9%)	32 (56.1%)	
Yes	45 (19.8%)	21 (46.7%)	24 (53.3%)	
What do you do to take care of your teeth before sleeping?				
No	198 (87.2%)	95 (48%)	103 (52%)	0.311
Yes	29 (12.8%)	11 (37.9%)	18 (62.1%)	
Do you like eating sweets?				
Always	70 (30.8%)	25 (35.7%)	45 (64.3%)	**<0.05**
Sometimes	157 (69.2%)	81 (51.6%)	76 (48.4%)	
Which of the bad habits listed below do you have?				
Biting nails	22 (9.7%)	7 (31.8%)	15 (68.2%)	
Bruxism	13 (5.7%)	8 (61.5%)	5 (38.5%)	0.356
Grinding	16 (7.1%)	7 (43.8%)	9 (56.3%)	
Nothing	176 (75.5%)	84 (47.7%)	92 (52.3%)	
How would you advise others to take care of your teeth?				
Brush your teeth regularly	119 (52.4%)	50 (42%)	69 (58%)	
Don't consume too much sweets	20 (8.8%)	13 (65%)	7 (35%)	
Go for regular dental visit	17 (7.5%)	9 (52.9%)	8(47.1%)	**<0.05**
Gurgling with salty warm water	29 (12.8%)	19(65.5%)	10 (34.5%)	
No advice	42 (18.5%)	15(35.7%)	27 (64.3%)	

Table 2 presents the influencing factors of respondent student's dental visits. The binary logistic regression variable namely age of the respondent, dental problem, gum bleeding, and the items the respondent used were found to influence the respondent student's dental visits where the p-value was less than 0.05.

From the binary logistic regression analysis, the students whose ages were 18-22 years had 0.242 times [AOR=0.242; 95% CI: 0.070-0.826] less dental visit history than the students whose ages were more than 25 years.

In cases where students who had dental cavities, dental caries, dental sensitivity, and toothache problems were still found to be 11.026 times [AOR=11.026; 95% CI: 3.747-32.440], 8.768 times [AOR= 8.768; 95% CI: 1.501-51.191], 3.615 times [AOR=3.615; 95% CI: 1.5-8.708], and 7.132 times [AOR=7.132; 95% CI: 2.021-25.163] more dental visits history compared to the students who didn't have any dental problems.

The students who had a gum bleeding problem while brushing had two times [AOR=2.133; 95% CI: 1.097-4.147] more likely to visit a dentist than the students who didn't have gum bleeding dental problems. The students who used toothpicks had 0.283 times [AOR=0.283; 95% CI: 0.121-0.654] fewer visits to a dentist than the students who used mouthwash.

Table 2: Binary logistic regression analysis for dental visiting history among students of Comilla University.

Variables	Adj. OR	95% CI for Adj. OR		P-value
		Lower	Upper	
Age of respondent				
18-22	0.242	0.070	0.826	**0.024***
23-25	0.569	0.174	1.865	0.352
>25	Ref.			
Faculty				
Science	0.980	0.437	2.198	0.962
Non-science	Ref.			
Which type of toothbrush do you use?				
Soft	0.167	0.017	1.660	0.127
Medium	0.194	0.019	1.972	0.166
Hard	Ref.			
Dental problem				
Dental Cavity	11.026	3.747	32.440	**<0.001****
Dental Caries	8.768	1.501	51.191	**0.016***
Tooth Sensitivity	3.615	1.500	8.708	**0.004***
Toothache	7.132	2.021	25.163	**0.002***
None of them	Ref.			
Do you have gum bleeding while brushing?				
Yes	2.133	1.097	4.147	**0.026***
No	Ref.			
Do you like eating sweets?				
Always	1.345	0.588	3.074	0.483
Sometimes	Ref.			
Do you fear the dentist's chamber?				
Yes	0.711	0.321	1.574	0.400
No	Ref.			
Which of the items mentioned below do you use?				
Dental floss	0.701	0.169	2.894	0.624
Miswak	0.426	0.177	1.021	0.056
Toothpick	0.283	0.121	0.654	**0.003***
Mouthwash	Ref.			
How would you advise others to take care of your teeth?				
Brush your teeth regularly	1.081	0.450	2.601	0.861
Don't consume too much sweets	0.944	0.224	3.976	0.938
Go for a regular dental visit	0.637	0.160	2.540	0.523
Gurgling with salty warm water	0.556	0.151	2.056	0.379
No advice	Ref.			

DISCUSSION

In our study, we find that 53.3% of university students have visited the dentist chamber for any reason, slightly higher than the European average of 50% [25]. Our research finding demonstrated that respondents did not show a significant relation between dental visits and gender ($p = 0.769$), which agrees with findings from studies on Indian postgraduate students [26]. On the other hand, a study carried out in Saudi Arabia indicated that compared to their male students, female students had considerably superior oral health-related knowledge, attitudes, and habits. The study's findings on the good oral health practices and attitudes of women may be explained by the fact that women tend to be more self-conscious about their looks. Women are therefore more likely to become knowledgeable about oral health and to visit a dentist [23]. A positive correlation has also been seen between dental visits and age [23]. Our research at the Comilla University demonstrated this relationship, with younger students seeing the dentist 0.242 times less often than older students. This suggests that older students have more oral health awareness than younger ones. A study performed at the University of Barcelona found a statistically significant association between the faculty and dental visits which shows that this variable had a relationship with the knowledge that the respondents have about oral hygiene [27]. A comparison of the respondents' oral health knowledge concerning toothbrush hardness ($p < 0.05$), and the interval between toothbrush replacements ($p < 0.05$) revealed no significant variations in this regard. When asked how often they brush their teeth, the majority of respondents (52.9%) said they only brush once a day (52.4%) and use a medium toothbrush. This is in contrast to a survey done at a military college in Bucharest, which revealed that 78.3% of respondents brushed their teeth twice a day (morning and evening) [28].

Research conducted by Peltzer and Pengpid [29] across 26 countries in Asia, Africa, and America involving 19,560 undergraduate students revealed that 67.2% of students brushed their teeth twice a day, 28.8% about once a day, and 4.0% never did. Studies in low- and middle-income nations (e.g., 52.2% in India, 35% in Lebanon, and 32% in Turkey) seem to have greater rates of students cleaning their teeth fewer than twice a day than children in high-income nations (7.9% in Italy or 25% in the United States). A study conducted on Zagreb's student body revealed that 83% of students, all of whom were Faculty of Dentistry students, brushed their teeth two to three times a day, and 17% stated they did so more than three times [30]. Students from Bjelovar, Croatia, presented an opposite statement [31].Over half (62.6%) of the participants swapped out their toothbrushes every four months. In a Military College in Bucharest poll, 53.8 percent of participants changed their brush every three months, and 34.3 percent once a month [28].In Zagreb, on the other hand, 48.3% of students use the same brush for fewer than three months [32].

According to numerous studies [9], [33], [34] the majority of students did not use dental floss. However, this is in contradiction with a study by Grewal and Kaur [35], which revealed that over half of American children regularly flossed their teeth. Furthermore, in contrast to some research, the majority of university students used mouthwash [9], [34], [36]. Additionally, we observe that students who use toothpicks have 0.284 times fewer visits than those who use mouthwash. Because miswak is used by Muslim students for cultural and religious reasons, university students should be instructed on how to use it properly and should be encouraged to practice excellent oral hygiene by brushing, using dental floss, and using toothpicks as preventative measures [33].

According to over half of university students,

individuals with gum disease attended dental chambers 2.133 times more often than students without gum disease. This result is in contradiction with the previous [36]-[38] carried out globally, which suggested using a toothbrush as a preventative care for gum disorders. One effective strategy to remove plaque and maintain healthy gums is to teach and encourage university students to wash their teeth regularly [33]. Eating sweets and fear of the dentist's chamber are significantly associated with dental visits. In our study, the students who consume sweets regularly face more dental problems and also visit the dentist's chamber more frequently. Besides, most of the students do not fear visiting a dentist's chamber. Dental visits are also significantly associated with dental problems, in our study, most of the students face tooth sensitivity problems, students who face dental cavities 11.024 times, dental caries 8.768 times, tooth sensitivity 3.615 times, toothache 7.132 times have more dental visits than none of them dental problem. Finally, we also find a significant association between advice and dental visits.

Our study revealed that the students advised to avoid too much sweet consumption, showed fewer dental visits. Diet provides sugars and other fermentable carbohydrates, which are metabolized to acids by plaque bacteria, resultant low pH favors the growth of the acidogenic and aciduric bacteria (*Mutans streptococci*) which subsequently produce dental caries. In terms of gargling with warm salty water, we find that respondents showed a significant reduction in dentist chamber visits. A similar correlation was reported by Karmawati *et al.*, highlighting that gargling with salt water, creates an alkaline environment that will inhibit the growth/proliferation of bacteria, and reduce toothache.

3.CONCLUSION

Oral hygiene is strongly correlated with an individual's attitude, behavior, and level of knowledge towards it. Acquiring adequate oral hygiene knowledge is essential for implementing effective oral hygiene practices. Even with education, a person's dental hygiene may still be inadequate if they do not exercise the right habits and attitude. Higher education students have a greater understanding of the significance of keeping good dental hygiene.

The data acquired from this research may be used by educational institutions to get a deeper understanding of student knowledge. As a result, they can enhance student education by offering a range of lectures or workshops. Conducting a study on student demographics across Bangladesh is highly recommended. In addition, a dental examination simultaneously is required to objectively evaluate the oral hygiene status on a regular basis.

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Consent for publication

Informed consent was obtained from every participant for documentation and examination.

Competing interests

The authors declare no competing interests.

Ethical approval

Ethical approval was granted by the Institutional Human Ethical Committee

Informed patient consent

All patients' clinical records were obtained with informed consent.

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DRUG-BASED INTERVENTIONS IN DENTISTRY: SUPPORT AND OPTIMIZATION TECHNIQUES

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ABSTRACT

Background: The introduction of modern approaches in dentistry requires a review of existing patient care strategies. The growing prevalence of non-communicable diseases (NCDs) in the structure of overall morbidity demonstrates the importance of considering comorbidities when planning dental interventions and medication support.
Objective: The study aims to systematise modern approaches to optimising the drug treatment of patients with NCDs in dental practice on the example of non-alcoholic fatty liver disease and its complications.
Methods: The research methodology included an analysis of modern scientific literature, statistical data and clinical studies.
Results: The relationship between oral diseases and non-alcoholic fatty liver disease is analysed. The pathophysiological mechanisms of this relationship and modern approaches to pharmacological support of patients are considered. Particular attention is paid to using herbal remedies and nutraceuticals in dental practice, which helps reduce systemic inflammation and the risk of complications.
Conclusion: The data obtained indicate the importance of an interdisciplinary approach in treating such patients.

***Keywords*:**periodontitis, surgical dentistry, therapeutic dentistry, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, herbal remedies, nutraceuticals.

1. INTRODUCTION

The modern paradigm of dental care for patients is based on a new understanding of the important role of oral health in maintaining human health and well-being.[1] At the stage of the dental industry transformation, much attention is paid to implementing the latest advances in pathobiology, regenerative medicine, bioengineering, and personalised medicine approaches.[1]

Diagnostics and treatment of oral diseases are among the most relevant areas of surgical dentistry. This area is relevant because of the significant prevalence of pathologies such as caries, periodontal disease, malignant tumours, and others, even in high-income countries.[2] According to Benzian et al.,[3] about 45% of the population, regardless of the region and economic development of the country, suffers from oral pathology, which often requires surgical intervention.

At the same time, the association of oral diseases with chronic non-communicable diseases (NCDs), in particular diseases of the gastrointestinal tract, has been proven[4, 5, 6], which requires the search for not only practical strategies for the treatment of dental diseases but also the development of multidisciplinary approaches to help patients with comorbidities.

The study aimed to systematise modern approaches to optimising the drug treatment of patients with NCDs in dental practice on the example of non-alcoholic fatty liver disease.

Literature review

Adequate pharmacological support in the provision of care to patients in the field of dental surgery is aimed at pain relief, prevention of complications and acceleration of healing processes in various types of pathological processes. It should be noted that, given the increasing proportion of the population with NCDs[7], the planning of pharmacological support for dental interventions should be carried out considering possible comorbidities individually for each patient.[1] In particular, the role of periodontal disease in the development of non-alcoholic fatty liver disease and its complication of non-alcoholic steatohepatitis has been confirmed, which, given the pathophysiological features of the course of this disease and its prevalence in the population, requires special attention to each patient.[6, 8, 9, 10, 11]

Since NCDs are often accompanied by impaired repair processes and immunodeficiency states,[12] and patients are intolerant to many chemicals and pharmacological agents (multiple-chemical sensitivity patients),[13, 14, 15] herbal remedies and probiotics are currently being considered as an alternative or complement to standard therapy.[16, 17, 18, 19]

METHODS

Study Design

This literature review was conducted in accordance with the PRISMA 2020 (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. The purpose of the review was to identify, assess, and synthesise peer-reviewed evidence related to pharmacological support in dental practice, particularly in patients with non-communicable diseases such as non-alcoholic fatty liver disease (NAFLD) and its complications.

Data Sources and Search Strategy

A comprehensive literature search was performed in the following databases: PubMed, Scopus, Web of Science, and Google Scholar, covering the publication period from January 2004 to December 2024. In addition, relevant clinical guidelines and consensus statements from professional medical associations and the World Health Organization (WHO) were reviewed.

The following keywords and their Boolean combinations were used:

[oral microbiome], [periodontitis], [dental pharmacology], [non-alcoholic fatty liver disease], [NAFLD], [steatohepatitis], [cirrhosis], [inflammation], [oxidative stress], [preventing], [oral hygiene practices].

The full search strategy for each database can be provided upon request.

Inclusion criteria:

– Peer-reviewed articles published between 2004 and 2024.

– Studies examining the relationship between oral health/dental interventions and NAFLD or other non-communicable diseases.

– Articles describing pharmacological or nutraceutical support in dental care.

– Guidelines or consensus documents on pharmacological strategies in dental practice.

– Articles written in English or Ukrainian (with translated versions).

Exclusion criteria:

– Abstracts without access to the full text.

– Case reports, editorials, conference abstracts, or letters to the editor.

– Studies unrelated to pharmacological or therapeutic aspects of dental care.

– Articles lacking clear methodological description or outcome measures.

Screening and Selection Process

The initial search yielded 250 articles. After duplicate removal and title/abstract screening, 72 full-text articles were assessed for eligibility. Of these, 32 articles met the inclusion criteria and were included in the final review. The article selection process is illustrated in the PRISMA flow diagram (Figure 1).

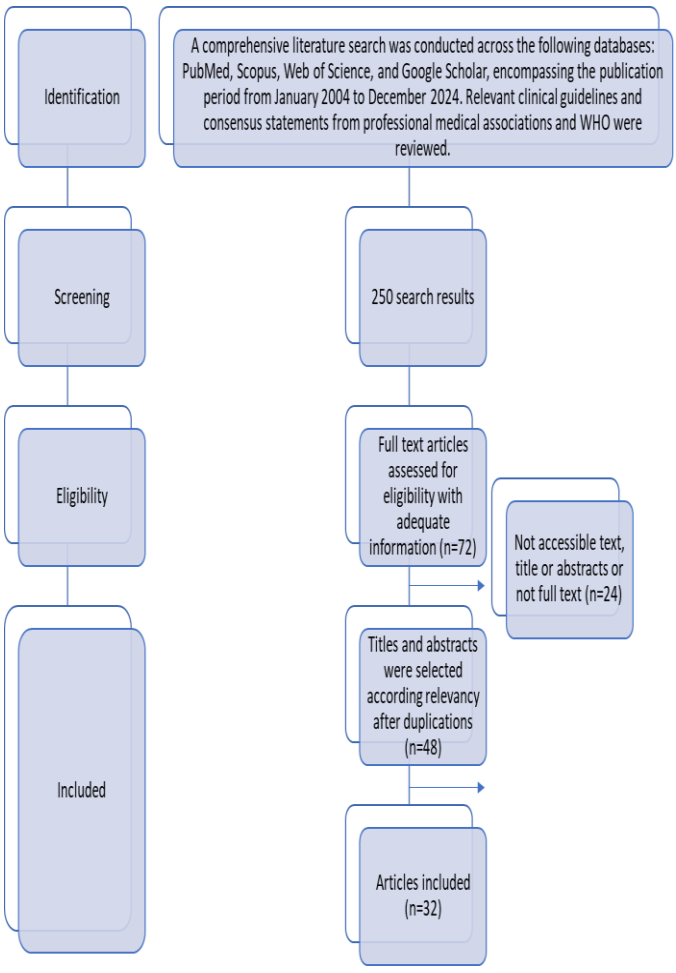


Figure 1. PRISMA 2020 Flow Diagram

Screening and eligibility assessment were performed independently by two reviewers. Discrepancies were resolved through discussion and consensus.

Data Extraction and Synthesis

A structured data extraction form was used to collect the following variables from each included article:

- Author(s) and publication year
- Study design and sample size (if applicable)
- Type of dental intervention
- Type of pharmacological support (e.g., antibiotics, nutraceuticals, herbal therapy)
- Main findings regarding efficacy or safety
- Relevance to NAFLD or comorbid conditions

Data were synthesised narratively due to the heterogeneity of study designs and outcomes. Quantitative synthesis (meta-analysis) was not performed.

Risk of Bias Assessment

Given the narrative nature of this review, a formal quantitative risk of bias tool was not applicable. However, methodological quality was assessed based on the following:

- Clarity of research objectives
- Transparency of methodology
- Sample size and design appropriateness
- Relevance of outcomes to the review objective
- Declaration of conflicts of interest and funding sources

Studies judged to have significant methodological limitations or lack of clarity in outcome reporting were excluded during the eligibility screening.

RESULTS

Features of drug treatment of patients with non-communicable chronic diseases in dental practice

The treatment of patients in dental practice involves the use of a variety of medications aimed at reducing symptoms, preventing complications and improving the quality of life of patients. Antibiotics are most often prescribed in dental practice, but according to,[20] as in the practice of family doctors, 80% of prescriptions are not appropriate in terms of both preventive and therapeutic effects.[21] Non-steroidal anti-inflammatory drugs, topical antibacterial drugs, toothpaste and rinses, herbal medicines and nutraceuticals are widely used.[16, 22]

One of the challenges that currently needs to be addressed is the intolerance of drugs and materials used in dental practice by patients with comorbidities. According to Cieplik et al.,[15] one-third of patients have complaints due to reactions associated with taking medications to treat the underlying disease. The most common side effects were detected in patients taking antihypertensive and psychotropic medications. Relevant diagnosed allergy to dental materials was found in 11.9% of patients with comorbidities. This indicates the need for interdisciplinary consultations when providing dental care to patients with comorbidities.

Patients' use of dental care differs for therapeutic and surgical interventions (Figure 2), although the main reasons for visits to a dentist are caries and periodontal disease.[22]

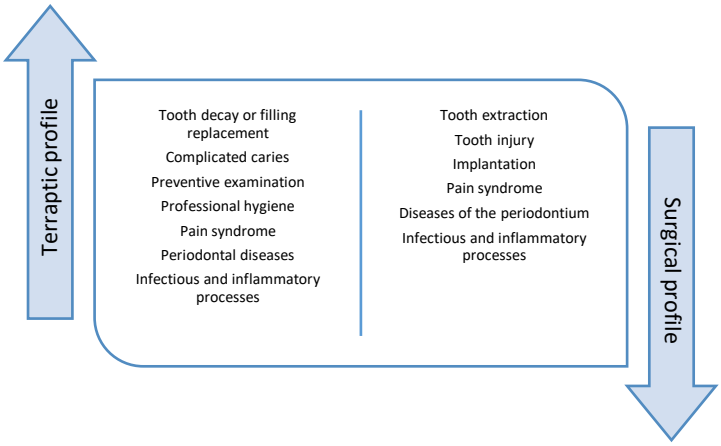


Figure 2. Reasons for seeking dental care in Ukraine, 2021
Source: author's drawing adapted from Mazur et al.[22]

The latest approaches to modern dental care make it possible for patients. In Table 1, we have systematised the main surgical interventions and options for medical support, taking into account modern advances in omics, conventional therapy, and nutraceutical therapy.

Table 1. Medication support after surgical interventions for oral diseases

Surgical intervention	Diseases / indications	Medication support
Tooth extraction	Terminal caries, periodontitis, tooth retention	Antibiotics (amoxicillin, clindamycin), NSAIDs (ibuprofen, ketorolac), probiotics for the prevention of dysbiosis
Atraumatic extraction	Implant planning, minimal tissue trauma	Biomaterials for socket preservation (collagen matrices, PRF-plasma enriched with growth factors), analgesics
Sinus lift	Bone atrophy of the upper jaw before implantation	Biomaterials (hydroxyapatite, β-TCF - beta-tricalcium phosphate), antibiotics, corticosteroids to reduce edema
Augmentation of bone tissue	Insufficient bone volume for implantation	Bioengineered materials (xenografts, bioactive glass, BMPs - bone morphogenetic proteins), antibiotics, analgesics
Dental implantation	Absence of teeth	Antibiotics, PRF (acceleration of osseointegration), probiotics for oral microbiota
Gingivectomy / gingivoplasty	Periodontitis, gingival hyperplasia	Antiseptics (chlorhexidine), regenerative gels with hyaluronic acid, laser therapy
Vestibuloplasty	Soft tissue correction to stabilise prostheses or implants	PRF, regenerative biomaterials, antiseptics
Bone grafting	Bone defects after injuries or tumours	Bioengineered materials (alloplastics, xenografts), PRF, BMP
Treatment of periapical lesions (root apex resection, cystectomy)	Chronic granulomas, cysts	Antibiotics, PRF, antioxidants (vitamin C, curcumin)
Laser surgery	Periodontitis, gingivitis, reduction of biofilm	Antiseptics, probiotics, photodynamic therapy
Mucogingival surgery (gum transplantation)	Gum recession, periodontitis	PRF, antiseptics, regenerative gels
Surgical treatment of peri-implantitis	Inflammation around implants	Bioactive coatings (antibacterial nanoparticles), probiotics, antibiotics

One area of dental care for the population is the treatment of periodontitis, which is the sixth most common disease among adults and is characterised by the destruction of tooth-supporting tissues, including cementum, periodontal ligament, and alveolar bone, due to inflammation.[10] Figure 3 shows the prevalence of severe forms of periodontal disease in the European region that required surgical intervention.

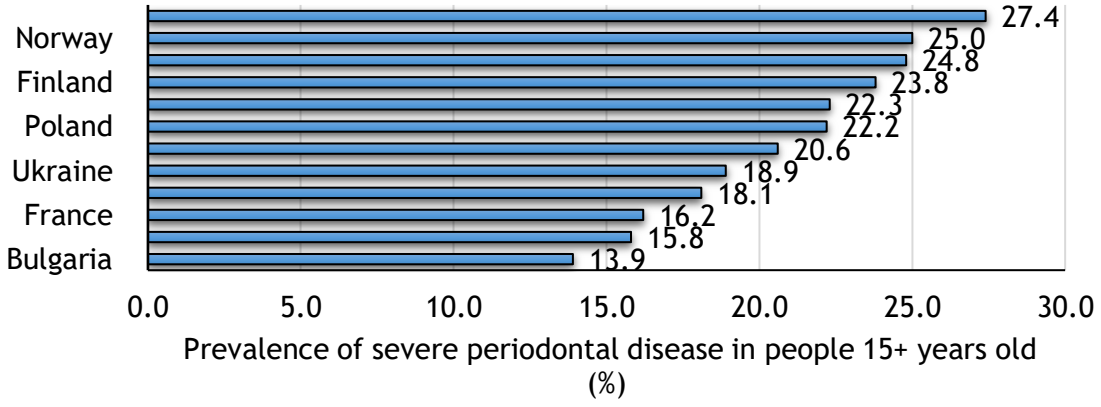


Figure 3. Prevalence of severe periodontal disease in the European region in people over 15 years of age, 2019
Source: author's drawing based on data from the WHO database[23]

Indications for the surgical treatment of periodontitis[16] include deep periodontal pockets that cannot be treated conservatively, destruction of alveolar bone, gingival detachment/gingival recession, granulation tissue and periodontal abscesses, loosening of teeth due to loss of bone support. The main surgical methods of periodontitis treatment include periodontal pocket curettage (closed and open), flap surgery for deep root cleaning and bone regeneration, regenerative surgery, gingivectomy/gingivoplasty, laser surgery – a minimally invasive approach to reduce inflammation and bleeding.

Studies have shown a link between periodontitis and NCDs, the pathophysiological causes of which are associated with metabolic disorders and the development of systemic inflammation,[11] which complicates the provision of dental care. Given the widespread prevalence of such diseases as non-alcoholic fatty liver disease, which occurs both in isolation and as a complication of diseases such as metabolic syndrome and diabetes mellitus, and the association of this disease with periodontitis, let us dwell on this in more detail.

Current understanding of the relationship between oral health and liver disease

Almost one in four people have NAFLD, and several hypotheses link NAFLD and periodontitis through periodontal pathogens, inflammatory mediators and oxidative stress.[11] Studies have demonstrated a statistically significant association between NAFLD and periodontitis, independent of socioeconomic variables, health habits, and the prevalence of metabolic syndrome. It has been established that both liver diseases can affect the state of the oral cavity by reducing local immunity, changing the composition of saliva and contributing to the development of periodontitis, and infectious processes in the oral cavity can contribute to the progression of liver disease by activating and maintaining systemic inflammation. Periodontal disease can negatively affect non-alcoholic fatty liver disease and lead to the development of severe and progressive forms such as non-alcoholic steatohepatitis and cirrhosis.[17]

There is evidence that oral dysbiosis alters the gut microbiota through the oral-intestinal axis, contributing to endotoxaemia and liver fibrosis.[11, 17] At the same time, it is known that the activation of fibrosis in the liver is also promoted by such oral pathogenic bacteria as P. gingivalis, T. denticola, and F. nucleatum, which are paradontal pathogens. In addition, patients with severe liver disease, such as non-alcoholic steatohepatitis and cirrhosis, have oral dysbiosis and pathological reproduction of flora that is opportunistic in people without such diseases, which complicates the treatment of such patients.[24]

The reason for this phenomenon is immune system dysfunction, which is the development of the underlying disease, which must be considered at the time of treatment. Although oral dysbiosis has been shown to influence the gut microbiome,[18] it increases the gut microbiome's permeability, and this leads to a complication of NAFLD, non-alcoholic steatohepatitis.

Hence, the modern approaches to treating patients with NCDs and primarily liver injury are planned to be combined with natural ingredients drugs.[25, 26] In the following subsection, we discuss how such drugs are prescribed.

Application of natural ingredient-based preparations in dental care

Often, after dental procedures, it is necessary to prescribe drugs to shorten the patient's recovery.[20] Due to the severe course of periodontal diseases in patients with NAFLD, especially non–alcoholic steatohepatitis and directly related pathogenesis of the intestine dysbiosis processes, it is necessary to use drugs that positively affect the main links of the pathological process and enhance tissue repair after surgical interventions.

With NCDs formed by the oral intestinal axis, considerable attention has been paid to the contribution of oral-based medicines to their treatment in the last few years.[18, 19]

This is a group of drugs based on the requirements of specific patients, including herbal medicines and pre-and probiotics.[19, 27] Table 2 systematises the primary drugs used in patients' current stage of dental care.

Table 2. Integrative approaches utilising phytopreparations, probiotics, and vitamins in dental patient care

Substance name	Effect	Diseases or surgical interventions
Phytopreparations		
Chamomile recutita (Chamomilla recutita)	Anti-inflammatory, antiseptic, soothing	Gingivitis, stomatitis, post-extraction period
Medicinal sage (Salvia officinalis)	Antiseptic, anti-inflammatory	Periodontitis, gingivitis, alveolitis
Calendula officinalis	Anti-inflammatory, wound healing	Stomatitis, postoperative wound healing
Oak bark (Quercus cortex)	Astringent, antiseptic, haemostatic	Gingivitis, periodontitis, bleeding gums
Propolis	Antibacterial, anti-inflammatory, immunostimulating	Periodontal disease, stomatitis, alveolitis, healing after tooth extraction
Aloe vera	Regeneration, anti-inflammatory	Mucosal burns, ulcerative stomatitis, postoperative recovery
Green tea extract (Camellia sinensis)	Antioxidant, anti-inflammatory, antibacterial	Prevention of caries, gingivitis, periodontitis
Vitamins		
Vitamin C (ascorbic acid)	Antioxidant, vascular strengthening	Periodontitis, bleeding gums, postoperative period
Vitamin D	Regulation of bone mineralisation	Osteoporosis, periodontal disease, dental implants
Vitamin A	Mucosal regeneration, antioxidant	Ulcerative stomatitis, healing after surgery
Vitamin E	Antioxidant, regenerating	Periodontal disease, dry mucous membrane
B vitamins (B1, B6, B12)	Neurotrophic effect, improved regeneration	Glossitis, stomatitis, facial nerve neuralgia
Rutin (vitamin P)	Strengthening of capillaries, anti-inflammatory	Bleeding gums, periodontitis, postoperative healing
Probiotics		
Lactobacillus reuteri	Antibacterial restoration of microflora	Prevention of caries, gingivitis, periodontitis
Bifidobacterium spp.	Anti-inflammatory, normalisation of the microbiota	Stomatitis, candidiasis, periodontitis
Streptococcus salivarius K12	Antibacterial protection against pathogenic microorganisms	Prevention of caries, periodontitis, stomatitis
Lactobacillus acidophilus	Restoring the balance of microflora, inhibiting the growth of pathogens	Oral dysbiosis, candidiasis, periodontitis

Using nutraceuticals, probiotics, and phytopreparat ions aims to immunomodulate by fighting the systematic inflammatory process stemming from infectious pathogenicity.[27] For example, administering nutraceuticals in non-alcoholic fatty liver disease decreases mitochondrial dysfunction, prevents oxidative stress development, and averts the progression of this disease to steatohepatitis.[25]

These are not traditional dental procedures or medical support, but they can be big assets in improving oral health and rehabilitation after surgery. They are also suggested as complementary or alternative approaches for patients who prefer such therapies and as part of a complete oral hygiene regimen.

Quertulin is used for the prevention and treatment of periodontal disease in patients with non-alcoholic steatohepatitis[28, 29] and has proven clinical efficacy.[28]

The results of the clinical trials based on the reduction of treatment time and dynamics were the indicators of the effectiveness of the proposed dosage forms of Quertulin as the therapeutic and prophylactic agent in the context of the treatment and prevention of initial and first forms of periodontitis on the background of non-alcoholic steatohepatitis. The drug is justified in clinical practice as a highly effective hepatoprotector with a broad angiotensin effect in treatment.[27, 28, 29]

DISCUSSION

The growing number of NCDs in morbidity structure suggests that as a response, dental interventions and medical support should be planned based on the possible comorbidities of patients.[23] Considering the relationship between oral diseases and NCDs, mainly non-alcoholic fatty liver disease and its complications, good oral care at an early stage can reduce the general development of complications or its severe course.[6, 9, 10, 28]. This is because of that between chronic inflammatory processes in the oral cavity (such as periodontitis and caries) and systemic inflammation that has a positive influence on the pathogenesis of NAFLD and steatohepatitis.[6, 9, 10]

Proven are the penetration of systemic bloodstream by oral bacteria and their metabolites and consequent induction of immune response disorders and elevated oxidative stress in the liver.[10] In particular, disruption of the oral microbiome can increase blood endotoxins such that the presence of metabolic syndrome or isolated, non-alcoholic fatty liver disease (NAFLD) results in steatohepatitis and cirrhosis.[17]

These relationships should be considered by the pharmacological support in the dental practice so that new therapeutic approaches emerge. For instance, the oral microbiome can be corrected by using anti-inflammatory drugs or probiotics to reduce peripheral and systemic inflammation, decreasing the chances of complications of NAFLD.[17] Additional promise implies using antioxidant drugs to lower the level of oxidative stress or immunomodulatory agents that can enhance the immune system's balance. Combined strategies involving the topical application of antimicrobials during dental care and general management for correcting metabolic disorders may be a more efficient approach for treating patients with concomitant dental diseases of other systems and organs.

Indeed, many questions are still open to further research. In particular, it is necessary to study in more detail the mechanisms of transporting oral bacteria to the liver, determine the optimal dental treatment methods for patients with NAFLD and evaluate the effectiveness of pharmacological interventions in preventing systemic complications. It is also important to develop interdisciplinary approaches to managing such patients, which involves close cooperation between dentists, gastroenterologists and hepatologists.[10, 11]

Current discussions also focus on the question of what is a greater risk for severe NCDs: oral diseases, metabolic disorders, lifestyle choices or socioeconomic factors[30, 31, 32]. Much attention is paid to heredity and genetic regulation of metabolic processes and susceptibility to infectious factors, as well as the integration of modern omics advances into dental education.[1, 30, 31, 32]

Summarising the above, it should be noted that drug support for dental patients at all stages of care should be comprehensive, considering the potential positive and negative systemic effects on the underlying disease and comorbidities.[16]

CONCLUSION

When providing dental care to patients, possible concomitant pathology and possible intolerance to pharmacological substances, especially when taking antihypertensive and psychotropic drugs, must be considered. Given the rise in antibiotic resistance, antibiotic prescriptions must be regularly monitored. Introducing integrated approaches to drug support based on natural remedies into clinical practice will help improve treatment effectiveness and reduce the risk of complications. Further research in this area promises to optimise treatment and reduce the risk of developing non-communicable chronic diseases associated with oral pathology.

DECLARATIONS

Financial support

This research did not receive any financial support.

Data Availability

All the data available upon request.

Ethics approval and consent to participate

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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THE EFFECT OF DIFFERENT RECONSTRUCTION PARAMETERS ON CONE BEAM CT IN THE ASSESSMENT OF TRABECULAR BONE MICROSTRUCTURE AT PLANNED DENTAL IMPLANT SITES

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ABSTRACT

Background: Cone beam computed tomography (CBCT) has become a valuable tool for evaluating trabecular bone microarchitecture before implant placement. However, CBCT-derived measurements may be influenced by reconstruction parameters such as voxel size and threshold level, potentially affecting the reliability of clinical assessments.

Objectives: This study aimed to evaluate the impact of CBCT reconstruction voxel size and threshold values on the measurement of trabecular bone microstructure to guide accurate image processing and interpretation.

Materials and Methods: Twenty-seven patients underwent CBCT scanning with a standard acquisition voxel size of 75 µm³. The images were reconstructed at voxel sizes of 75, 115, and 150 µm³ to assess the influence of reconstruction resolution. Additionally, the effect of threshold variation was examined by modifying the global threshold value by ±15%. Trabecular parameters including bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp) were analyzed using CTAnalyser software. Statistical evaluation was performed using one-way ANOVA with Tukey's post hoc test ($p < 0.05$).

Results: Reconstruction voxel size had no significant effect on BV/TV, Tb.Th, or Tb.Sp, but Tb.N differed significantly between the 75 µm and 150 µm voxel sizes ($p = 0.010$). In contrast, threshold variation significantly affected all trabecular parameters ($p < 0.001$), with all pairwise comparisons showing statistical significance.

Conclusion: While CBCT reconstruction voxel size showed limited influence on trabecular metrics, threshold level had a major effect on all measured parameters. These findings emphasize the importance of threshold standardization in clinical CBCT analysis.

Keywords: CBCT, trabecular bone, voxel size, threshold value, bone microstructure, image reconstruction

1. INTRODUCTION

Evaluating the trabecular bone microarchitecture using cone beam computed tomography (CBCT) images prior to implant placement is an increasingly adopted approach in treatment planning, aimed at enhancing the success rate and long-term stability of dental implants [1]. This approach is warranted because the microstructural characteristics of trabecular bone have been demonstrated to significantly influence primary stability at the time of implant placement [2, 3]. Moreover, the condition of the trabecular bone microarchitecture prior to implant placement serves as a reliable predictor of primary implant stability [4]. However, the evaluation of trabecular bone parameters from CBCT images may be

affected by the reconstruction settings employed during image processing, including reconstruction parameters such as voxel size and threshold values.

The reconstruction voxel size refers to the voxel dimensions applied during the image reconstruction process from raw data. Typically, a larger reconstruction voxel size than that used during image acquisition is selected to decrease reconstruction time, minimize computational demands and reduce the radiation dose[5]. This approach is commonly employed in certain applications, such as the analysis of large-scale finite element models derived from micro-CT-based imaging [5-7]. However, it is generally assumed that utilizing a reconstruction voxel size larger than the acquisition voxel size may lead to a reduction in image accuracy [8]. Previous investigations into the effects of CBCT reconstruction voxel size have predominantly addressed its influence on image quality [9] and the three-dimensional assessment of dental morphology [10]. However, the influence of reconstruction voxel size on the measurement of trabecular bone microstructure remains insufficiently understood.

Segmentation refers to the process of distinguishing bone or soft tissue from surrounding structures in imaging, a procedure that can be influenced by the selected threshold values [11]. Numerous studies have employed micro-computed tomography (μCT) to examine the impact of threshold values on trabecular microstructural parameters, including trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp), and bone volume fraction (BV/TV), particularly in small animal models [12]. While numerous studies have explored scanning parameters in μCT, research on the influence of reconstruction parameters in CBCT remains limited. Given the emerging use of CBCT for assessing trabecular bone to support surgical treatment planning, it is essential to investigate how CBCT reconstruction parameters affect these measurements. Thresholding is widely employed for image segmentation due to its simplicity and practicality, particularly in bone volume measurements [13]. Consequently, this study also examines the impact of threshold values on the quantification of trabecular bone microstructure.

OBJECTIVES

This study aimed to evaluate how different CBCT image reconstruction settings specifically, the threshold value and voxel size affect the analysis of trabecular bone microstructure.The goal is to offer clinicians evidence-based recommendations for selecting appropriate voxel sizes and threshold settings when reconstructing CBCT images for accurate assessment of trabecular bone features.

MATERIAL AND METHODS

27 patients were referred from the oral and maxillofacial surgery clinic, Faculty of dentistry MSA university, Giza, Egypt to take a pre implant CBCT scan (Medically free male or female patients,age ranging from 20 to 40 years) any patient with developmental anomalies or pathological conditions affecting the edentulous area were excluded from the study(Fig.1). The study protocol was approved by the Ethics Committee of fucalty of dentistry cairo university, and informed consent was obtained from all participants.



Figure 1. NewTom Gino HR CBCT Machine

Every Patient was imaged once using CBCT System (NewTom Gino HR) Fig (1) at Oral and Maxillofacial Radiology department. Faculty of Dentistry, MSA University. The CBCT scanning parameters employed in this study included a voxel size of 75 μm³, a field of view (FOV) of 6 × 6 cm, and a 360° arm rotation. Image acquisition was performed at 70 kV, 8 mA, and an exposure time of 11.3 seconds. Later on, another 2 volumes were reconstructed with voxel size 115, 150 μm³ respectively. The patients' heads were positioned where the mid-sagittal plan perpendicular to the floor and the Frankfort plane was parallel to the floor. The acquired images were converted to DICOM format and imported into DataViewer (version 1.5, SkyScan, Kontich, Belgium) to generate Axial views for subsequent image processing and analysis using CTAnalyser software (version 1.15, SkyScan, Kontich, Belgium).

Image reconstruction was performed using three different voxel sizes 75, 115 and 150 μm³ resulting in three distinct datasets Fig (2). For the initial dataset, a region of interest (ROI) measuring 14 × 8 mm was selected for the reconstructed volume at a voxel size of 75 μm³. The identical ROI was subsequently used for the reconstructed remaining two datasets with voxel sizes of 115 μm³ and 150 μm³, respectively (Fig. 2). Accordingly, three sets of ROIs were obtained, each of which was thresholded and binarized using a global threshold value automatically generated by CTAnalyser software (version 1.15, SkyScan, Kontich, Belgium).

This automatically generated global value was adopted as the optimal threshold for each patient in the study. Subsequently, trabecular bone analysis was conducted using the same software to evaluate the influence of varying reconstruction voxel sizes on the measurement of trabecular bone parameters, including trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp), and bone volume fraction (BV/TV

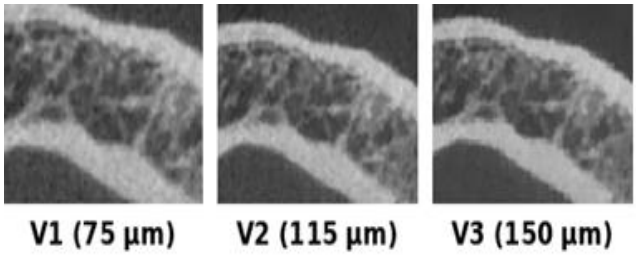


Figure 2. Three reconstructed images using voxel size of 75 µm3 (V1), 115 µm3 (V2) and 150 µm3 (V3)

To evaluate the effect of varying threshold values on the CBCT datasets, the first dataset with voxel size 75 µm³ was used as the baseline. Three datasets were generated by adjusting the global threshold value as follows: Dataset A with the original automatically generated global value and was used as a reference, and Datasets B 15% decrease and C with a 15% increase relative to the original global threshold value (Fig. 3). All three datasets were imported into CTAnalyser software (version 1.15, SkyScan, Kontich, Belgium) for ROI selection. Subsequently, three dimensional analyses were conducted to assess trabecular bone parameters, including trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp), and bone volume fraction (BV/TV).

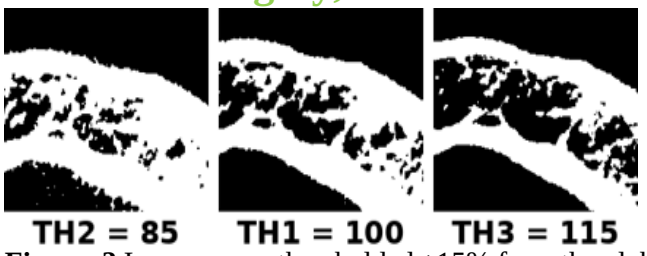


Figure. 3 Images were thresholded ±15% from the global threshold value (85, 100: global threshold value and 115) and binarised in the CTAnalyser software

STATISTICAL ANALYSIS

All statistical analyses were performed using the Microstat7 for Windows statistical package (Microstat Co). The normality of the data was confirmed using the Kolmogorov–Smirnov test, which showed no significant deviation from a normal distribution (D = 0.11226, p = 0.84844). One-way analysis of variance (ANOVA) was conducted to evaluate the effect of voxel size (75 µm, 115 µm, 150 µm) and threshold level (+15%, global, –15%) on trabecular bone microstructure parameters including bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp). When significant differences were found, post hoc comparisons were conducted using Tukey's test. A p-value < 0.05 was considered statistically significant.

RESULTS

Effect of Voxel Size

No statistically significant differences were observed in bone volume percent (BV/TV), trabecular thickness (Tb.Th), or trabecular separation (Tb.Sp) among the three voxel sizes (p = 0.860, 0.184, and 0.078, respectively). However, trabecular number (Tb.N) showed a statistically significant difference between the 75 µm and 150 µm voxel sizes (p = 0.010), indicating sensitivity to voxel resolution for this parameter. Table 1

Table 1. Effect of Voxel Size on Trabecular Parameters

Parameter	75 µm (Mean ± SD)	115 µm (Mean ± SD)	150 µm (Mean ± SD)	p-value
Bone Volume Percent (BV/TV %)	52.25 ± 22.42	49.41 ± 22.50	49.06 ± 24.88	0.860
Trabecular Thickness (µm)	534.3 ± 166.15	554.71 ± 163.10	616.52 ± 177.77	0.184
Trabecular Number (1/µm)	0.00095 ± 0.0002	0.00085 ± 0.0003	0.00076 ± 0.0003	0.015
Trabecular Separation (µm)	540.02 ± 180.63	597.55 ± 188.22	658.79 ± 201.10	0.078

Effect of Threshold Level

Threshold variation significantly influenced all trabecular bone parameters. Statistically significant differences ($p < 0.001$) were found in bone volume percent, trabecular thickness, trabecular number, and trabecular separation across all three threshold levels (+15%, global, –15%). Post hoc Tukey tests confirmed that all pairwise comparisons were statistically significant ($p < 0.001$), indicating a high sensitivity of these measurements to threshold selection. Table 2

Table 2. Effect of Threshold on Trabecular Parameters

Parameter	+15% (Mean ± SD)	Global (Mean ± SD)	-15% (Mean ± SD)	p-value
Bone Volume Percent (BV/TV %)	20.88 ± 10.22	40.61 ± 14.53	66.14 ± 15.08	<0.001
Trabecular Thickness (µm)	404.85 ± 95.87	517.33 ± 115.77	680.54 ± 143.37	<0.001
Trabecular Number (1/µm)	0.0005 ± 0.0002	0.0008 ± 0.0002	0.0010 ± 0.0002	<0.001
Trabecular Separation (µm)	875.55 ± 149.59	718.89 ± 156.97	494.59 ± 140.89	<0.001

DISCUSSION

Trabecular bone plays a central role in bone metabolism due to its high turnover rate [2], making its microstructural assessment crucial for evaluating bone quality. µCT is widely recognized as the gold standard for such assessments because of its proven accuracy and reproducibility [14]. However, its application in clinical settings is limited, as it requires invasive bone removal and cannot be used for longitudinal assessment in living patients.

In recent years, CBCT has emerged as a promising alternative. Multiple studies have demonstrated that CBCT provides valid and reliable assessments of trabecular bone microstructure when compared with µCT [15–18]. Tayman et al. reported excellent agreement between µCT and CBCT in assessing trabecular parameters, although they noted potential discrepancies due to differences between in vivo and ex vivo bone samples [19]. Notably, the current study is the first to investigate these parameters in vivo in human patients, providing clinically relevant insights.

Previous research by Aso et al. using a sheep head model found no significant effect of CBCT reconstruction voxel size on trabecular measurements but called for further investigation using human data [20]. Additionally, it has been shown that while scanning parameters (e.g., voxel size, FOV, rotation) are known to influence CBCT output [21], the impact of reconstruction parameters such as voxel size and threshold level remains underexplored, particularly in human subjects [22, 23].

In this study, CBCT images acquired at a scanning voxel size of 75 µm³ were reconstructed at 115 µm³ and 150 µm³. The results revealed no significant differences in bone volume percent (BV/TV), trabecular thickness (Tb.Th), or trabecular separation (Tb.Sp). A significant difference was only observed in trabecular number (Tb.N) between 75 µm³ and 150 µm³, suggesting limited sensitivity of most parameters to changes in reconstruction voxel size. These findings align with Aso et al.'s results on animal models and with prior work on mandibular cadaveric samples, except for Tb.N [20, 24].

Interestingly, our results contrast with earlier µCT studies, which indicated that BV/TV and Tb.Th are susceptible to reconstruction voxel changes when images scanned at 21 µm³ were reconstructed at 50 or 110 µm³ [8, 25]. This discrepancy may be attributed to the higher original scanning resolution used in µCT compared to CBCT. The influence of voxel size is more pronounced when the difference between scanning and reconstruction resolution is substantial, as shown previously [8, 25].

In contrast to voxel size, threshold level had a significant impact on all trabecular bone parameters in our study. Lowering or increasing the threshold by 15% from the global value significantly altered BV/TV, Tb.Th, Tb.N, and Tb.Sp. These findings corroborate prior research highlighting the sensitivity of threshold selection in trabecular analysis using µCT [26, 27]. Our results also partially agree with Aso et al.'s findings; while both

studies observed changes upon lowering the threshold, our study found significant differences with increased thresholds as well. This discrepancy may stem from the in vivo nature of our data, as bone microstructure and mineral concentration may differ in living versus post mortem tissue.

Some variability in measurement may also be due to partial volume effects, which can blur the boundary between trabeculae and surrounding voxels, especially at lower resolutions [28]. Although some studies suggest that Tb.N is less sensitive to threshold variations within realistic limits, our findings showed consistent significant changes across all threshold levels tested.

There are, however, limitations to acknowledge. µCT was not used for validation in this study due to its invasive nature and computational demands. Despite this, numerous studies have shown strong correlations between CBCT and µCT for trabecular measurements [17, 29], supporting the reliability of CBCT in this context.

Further studies comparing different CBCT reconstruction settings are recommended to enhance the validity of these findings and support the clinical application of CBCT in evaluating trabecular bone microstructure.

Conclusion

This study demonstrated that variations in CBCT reconstruction voxel size had minimal impact on trabecular bone microstructure measurements, with only trabecular number (Tb.N) showing sensitivity between the smallest and largest voxel sizes. In contrast, threshold level significantly affected all evaluated parameters, indicating its critical role in image segmentation and analysis, among the metrics evaluated, trabecular number appears to be the most consistent and least affected by threshold variations. These findings highlight the importance of standardized threshold selection in clinical assessments using CBCT. Further research involving different CBCT systems and reconstruction protocols is recommended to confirm the generalizability of these results in broader clinical settings.

Author Contributions

- **Mo Gamal:** A- research concept and design; B – collection and\or assembly of data; C – data analysis and interpretation; D – writing the article; F – final approval of article
- **Gihan Omar:** A – research concept and design; B – data analysis and interpretation; E – critical revision of the article; f – final approval of the article
- **Hany Omar:** B – data analysis and interpretation; E – critical revision of the artical; F – final approval of artical
- **Ahmed Mohamed Hossam:** B – collection and\or assemply of data; E – criticle revision of the article; F – final approval of article

DECLARATIONS

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Competing interests

The authors declare no competing interests.

Ethical approval

Institutional review board statement: The study protocol was approved by the Ethics Committee of fucalty of dentistry Cairo university, Egypt (Approval Number: 6/7/23)

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STRUCTURED AIRWAY MANAGEMENT AND EVIDENCE-BASED EXTUBATION STRATEGY FOR LUDWIG'S ANGINA

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ABSTRACT

Ludwig's angina is a rapidly advancing infection affecting the submandibular, sublingual, and submental spaces, posing a significant risk of airway obstruction. Prompt airway management and an appropriate extubation strategy are essential to prevent life-threatening complications. We report on a 20-year-old male patient who developed worsening neck swelling, swallowing difficulties, and respiratory issues as a result of untreated dental cavities. Upon examination, submandibular cellulitis and posterior tongue displacement were observed. Laboratory results showed leukocytosis, thrombocytopenia, and hypoalbuminemia, indicating a systemic inflammatory response. Based on the clinical and laboratory evidence, a diagnosis of Ludwig's angina complicated by sepsis was established. To ensure airway patency, awake fiber-optic intubation was conducted, followed by the commencement of mechanical ventilation. Intravenous administration of ceftriaxone and metronidazole was employed as empirical therapy to address both aerobic and anaerobic bacterial infections. The patient received care in the intensive care unit (ICU), where continuous monitoring and sedation were maintained. On the eighth day, extubation was successfully performed after confirming a positive cuff leak test, the absence of further edema, and stable respiratory function. The patient achieved full recovery without necessitating a tracheostomy or additional corticosteroid treatment. This case study demonstrates the successful conservative management of Ludwig's angina in a young adult, facilitated by prompt airway intervention, comprehensive multidisciplinary care in the intensive care unit (ICU), and a carefully planned extubation strategy. It emphasizes the critical role of personalized, evidence-based protocols in addressing complex airway infections and supports the application of non-surgical methods when guided by objective criteria.

***Keywords*:** Ludwig's angina, airway management, fiber-optic intubation, extubation strategy, sepsis

1. INTRODUCTION

Ludwig's angina is a swiftly advancing, potentially fatal infection that affects the sublingual, submental, and submandibular regions, often stemming from dental infections. This condition leads to widespread cellulitis and swelling in the neck and the floor of the mouth, significantly increasing the risk of airway obstruction due to tongue displacement and edema.[1] Despite progress in medical treatments, the condition continues to have a high mortality rate, mainly due to airway compromise or the spread of infection to the mediastinum.[2]

Effective management of the airway is vital to prevent respiratory distress and death. However, the presence of extensive edema and altered anatomy complicates standard intubation procedures, often necessitating advanced methods such as fiber-optic intubation or tracheostomy for securing the airway.[3] In such cases, extubation requires meticulous evaluation to prevent complications like reintubation or respiratory failure.[4]

This report presents the case of a young adult with Ludwig's angina resulting from untreated dental caries. It underscores a systematic approach to postoperative airway management and extubation, highlighting the importance of criteria-based protocols to ensure patient safety. Through this case, we aim to demonstrate the essential role of timely, evidence-based interventions in enhancing outcomes for patients facing complex airway challenges.[5]

2.CASE PRESENTATION

A 20-year-old male with untreated dental caries in the right lower molar presented with progressive neck swelling, dysphagia, and respiratory distress over ten days. He developed fever and purulent neck discharge a day before admission.

On examination, he was febrile and tachypneic with oxygen saturation of 98% on nasal cannula. He had bilateral swelling, erythema, and tenderness extending to the clavicle, accompanied by posterior tongue displacement due to sublingual edema, indicating imminent airway obstruction. Figure 2 illustrates the patient's preoperative condition with severe submandibular swelling and erythema and postoperative improvement following therapy.

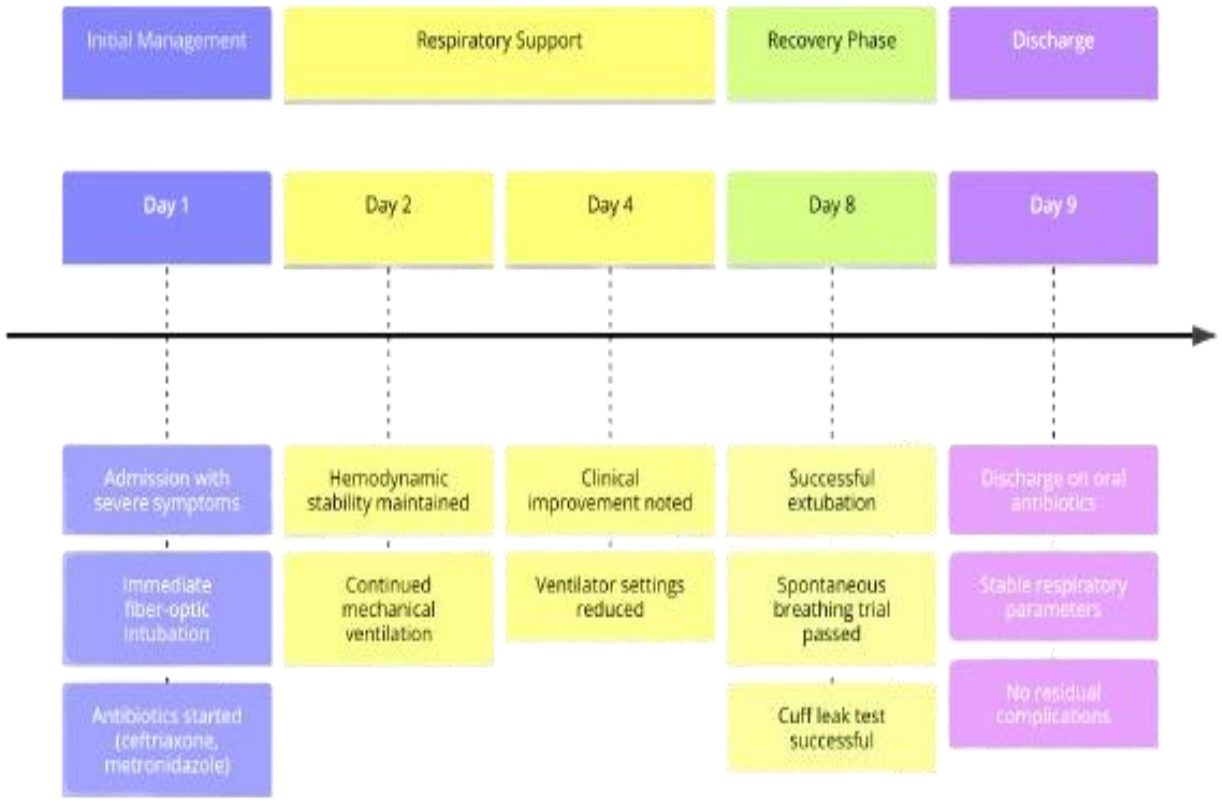


Figure 1. Case Report Timeline



Figure 2. Preoperative (Left) and Postoperative (Right) Images of a Patient with Ludwig's Angina

Clinically, increased respiration rate >22 breaths/minute alone cannot fulfil sepsis suspicion as it scored 1 on qSOFA score. But laboratory result revealed leukocytosis, thrombocytopenia, reduced p/f ratio of 300, and elevated level of both creatinine and bilirubin level. This finding supported the diagnosis of sepsis as it scored 5 in Sequential Organ Failure Assessment (SOFA) along with clear site of infection. The patient was diagnosed with Ludwig's angina complicated by sepsis. Preoperative neck radiographs (see Figure 3) confirmed submandibular swelling and airway narrowing, while chest radiographs excluded mediastinal spread or pulmonary involvement.

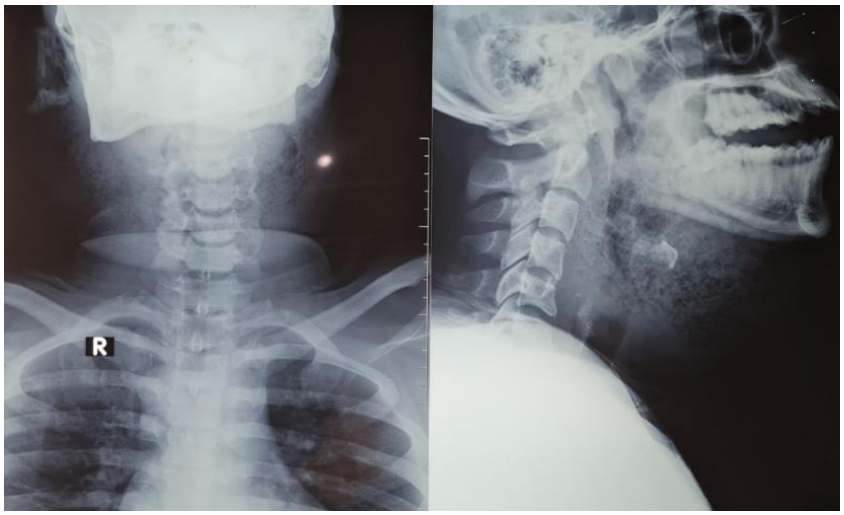


Figure 3. Preoperative Lateral and Anteroposterior Radiographs Showing Submandibular Soft Tissue Swelling in Ludwig's Angina

Patient then planned to have urgent surgical incision and abscess drainage. Airway management was initiated using awake fiber-optic intubation, deemed safest due to extensive edema and anatomical distortion. The patient was placed on mechanical ventilation and started on intravenous ceftriaxone and metronidazole to target both aerobic and anaerobic pathogens. Empirical antibiotic selection was based on literature showing high efficacy against Streptococcus viridans, Staphylococcus aureus, and anaerobic oral flora, which are commonly involved in Ludwig's angina. Streptococcus constellatus and Porphyromonas asaccharolytica were identified from abscess's tissue culture, and both were sensitive to ceftriaxone and metronidazole, which then continued for the next seven days.

Continuous intensive care unit monitoring was performed, including invasive blood pressure measurement, electrocardiography, pulse oximetry, and serial arterial blood gas analyses. Hemodynamic status, urine output, and neurological function (via GCS) were monitored every 2–4 hours. Ventilator parameters, including tidal volume, PEEP, FiO_2, and peak airway pressure, were adjusted daily.

Sedation was maintained using intravenous midazolam (2–5 mg/hour) and fentanyl infusion (30-50 mcg/hour) with Richmond Agitation Sedation Scale (RASS) targets of -1 to -3 to ensure patient-ventilator synchrony while avoiding oversedation. Opioids were given for the first 24 hours then continued with intravenous ibuprofen and paracetamol. Daily sedation interruption was performed to assess neurological recovery.

On the second day of admission, the patient remained hemodynamically stable. Although neck swelling persisted, it showed no progression. The patient was managed with intravenous fluids and head elevation to reduce airway edema. No diagnostic or logistical challenges were encountered.

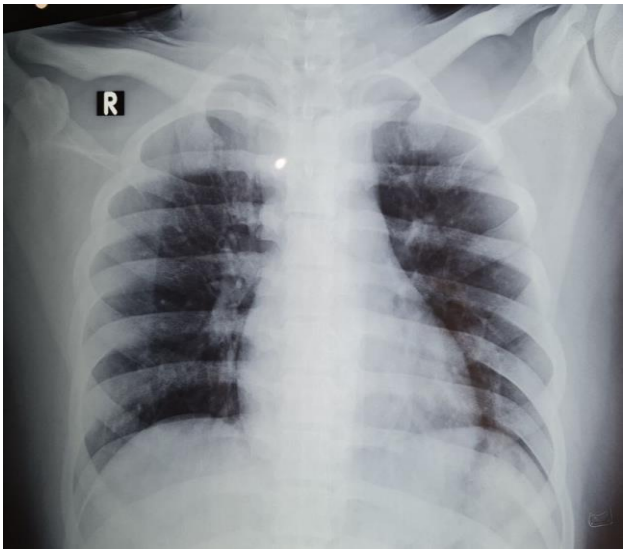


Figure 4. Chest Radiograph Showing Normal Lung Fields and Mediastinum in a Patient with Ludwig's Angina

By the fourth day, infection signs improved, allowing gradual reduction of ventilatory support with oxygen saturation stable at 97%. Daily airway and ventilatory assessments guided a smooth weaning process without complications.

On the eighth day, a successful cuff leak test confirmed minimal airway edema, supporting extubation readiness. The extubation strategy was based on a multi-criteria protocol including: improvement in inflammatory markers, adequate mental status (GCS 15), ability to tolerate spontaneous breathing trials for more than 2 hours with minimal ventilator support ($FiO_2 \leq 0.4$, PEEP $\leq$ 5 cmH_2O), and absence of stridor or neck swelling progression.

The procedure was conducted in a semi-upright position under close ICU supervision, with difficult airway cart and reintubation equipment prepared at the bedside.

Extubation was performed in a controlled setting with supplemental oxygen. Post-extubation monitoring included continuous pulse oximetry, respiratory rate, and voice quality assessment to detect early signs of airway compromise. Post-extubation, the patient's respiratory parameters remained stable, with an oxygen saturation of 97% on room air.

By the ninth day, neck swelling had resolved, and the patient resumed normal oral intake. He was discharged on oral antibiotics with otolaryngology follow-up. Complete recovery was achieved with no residual symptoms, emphasizing the importance of timely airway management and structured extubation protocols in complex infections like Ludwig's angina. Post-extubation photographs were not obtained due to the rapid improvement in the patient's condition and early discharge, which limited the opportunity for standardized postoperative documentation.

3. DISCUSSION

This case highlights the critical importance of timely airway management and structured extubation criteria in Ludwig's angina, a rapidly progressing and potentially fatal infection involving diffuse cellulitis of the mouth floor.[3] In this patient, an untreated molar infection spread to the sublingual, submandibular, and submental spaces, leading to airway compromise, dysphagia, and respiratory distress. The ability of Ludwig's angina to infiltrate fascial planes and potentially cause asphyxia or mediastinal spread underscores its high mortality risk.[6]

Awake fiber-optic intubation was employed in this case, adhering to guidelines for managing anticipated difficult airways. This approach allowed visualization of the airway while maintaining spontaneous breathing, thus minimizing the risk of exacerbating edema associated with general anesthesia.[5] Evidence consistently supports fiber-optic intubation in Ludwig's angina, as it reduces complications by avoiding excessive airway manipulation and provides clear anatomical guidance.[7,8] Although technically demanding, its benefits in high-risk cases, as demonstrated here, underscore its value in improving outcomes.

The decision to forgo steroid use in this case reflects ongoing debate in Ludwig's angina management. While steroids have been suggested to reduce inflammatory edema and improve airway patency, evidence supporting their efficacy remains limited. A narrative review analyzing 31 cases noted that dexamethasone was often used without significant adverse effects, yet concerns persist that steroids may mask infection progression.[9,10] Given this uncertainty and the favorable clinical progress achieved without steroids in this patient, a conservative management strategy relying on antibiotics and mechanical ventilation was deemed appropriate.

Broad-spectrum antibiotic therapy is a cornerstone of Ludwig's angina treatment, targeting the mixed aerobic and anaerobic pathogens typically involved. Ceftriaxone and metronidazole were selected for their efficacy against common causative organisms, including Streptococcus viridans and Staphylococcus aureus.[11] Early initiation of antibiotics is essential to controlling infection, mitigating systemic inflammation, and preventing complications such as mediastinal spread. The literature underscores the importance of aggressive antibiotic regimens, as inadequate treatment can escalate the condition and necessitate invasive interventions like tracheostomy.[6] In this case, timely antibiotic therapy effectively controlled the infection without additional surgical drainage.

Extubation in Ludwig's angina presents unique challenges due to the risk of residual airway edema and potential complications such as stridor or obstruction. The cuff leak test, which assesses airway patency by evaluating air escape around the deflated endotracheal tube cuff, was instrumental in determining extubation readiness in this patient.[12] Studies suggest that a cuff leak volume below 110–130 mL indicates a higher risk of post-extubation stridor.[11] The positive cuff leak test in this case provided objective confirmation of minimal edema, supporting the decision to proceed with extubation. In addition to the cuff leak test, the extubation protocol in this case followed the recommendations by the Society of Critical Care Medicine (SCCM) and the Difficult Airway Society (DAS), which emphasize readiness criteria such as stable hemodynamics, absence of stridor, sufficient spontaneous breathing capacity, and access to reintubation tools. Structured extubation protocols, as applied here, align with evidence-based recommendations for reducing post-extubation complications in high-risk patients.[13]

Balancing timely extubation with ensuring airway stability is critical. Prolonged intubation increases risks such as ventilator-associated pneumonia, whereas premature extubation can lead to respiratory failure.[14,15] In this case, extubation was delayed until the eighth day to allow sufficient resolution of edema and infection. This decision, coupled with gradual weaning and daily spontaneous breathing trials, facilitated a smooth transition from mechanical ventilation to spontaneous breathing.[1,16] Protocolized weaning processes have been shown to improve outcomes in patients with difficult airways, providing a controlled environment for assessing respiratory function and stability.

This case demonstrates the benefits of a comprehensive, criteria-based approach to airway management and extubation in Ludwig's angina. The combination of fiber-optic intubation and objective extubation readiness testing minimized risks and contributed to a favorable outcome. Close monitoring of hemodynamic and respiratory parameters allowed timely adjustments in ventilatory support, ensuring patient safety throughout the recovery process.[17]

Despite these strengths, certain limitations are acknowledged. Fiber-optic intubation, while recommended, requires specialized equipment and trained personnel, which may not be readily available in all settings.[5] In such cases, alternative techniques like tracheostomy might be necessary, potentially increasing morbidity. Additionally, although the cuff leak test provided valuable insights into airway patency, it is not infallible; undetected edema could still pose risks for post-extubation complications, emphasizing the need for clinical judgment alongside objective measures.[18]

Compared to similar reports, this case is notable for the patient's young age, the successful avoidance of tracheostomy, and the complete resolution of infection without corticosteroid use. While most published cases of Ludwig's angina requiring ICU care involved older adults or tracheostomy interventions, this case offers a rare example of full recovery through structured, non-surgical, protocol-driven critical care.[19]

Moreover, our management was consistent with the Infectious Diseases Society of America (IDSA) guidelines for severe soft tissue infections, which advocate for early airway control, appropriate empiric antibiotics, and close monitoring in ICU settings. Our extubation strategy followed SCCM and DAS principles, reaffirming the value of multidisciplinary, guideline-based care in complex airway infections.[20]

This case not only reinforces existing knowledge but adds practical insight for ICU clinicians managing high-risk airway scenarios. The structured protocol applied in this young adult resulted in complete recovery, suggesting that when appropriate resources and training are available, non-invasive strategies can yield excellent outcomes.[21]

4.CONCLUSION

This case highlights the successful management of Ludwig's angina using a structured airway strategy, including awake fiber-optic intubation and objective extubation criteria. Full recovery was achieved without tracheostomy or corticosteroids, emphasizing the value of guideline-based, non-invasive ICU care in complex airway infections.

5.DECLARATIONS

Ethics approval and consent to participate

This case report was conducted in accordance with institutional ethical standards. Written informed consent was obtained from the patient for the publication of this case and accompanying clinical data. Ethical approval was not required, as the report describes a single clinical case without experimental intervention.

Consent for publication

All relevant clinical data are included within the article. Additional information is available from the corresponding author upon reasonable request.

Competing interests

The authors declare no competing interests.

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CASE REPORT

PRECISION IN PERIODONTICS CUSTOMIZED TREATMENT PLANNING IN MULTI-TOOTH PERIODONTAL REHABILITATION- A CASE REPORT

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The primary goal of periodontitis treatment is to eliminate the causes, reduce modifiable risk factors and bring about periodontal regeneration. The treatment of Periodontitis requires a customized treatment plan for multiple tooth periodontal rehabilitation. Choice of appropriate treatment strategy improves the overall success rate of procedures and improvement in the periodontal status of the patient. The present case report describes a 23-year-old male patient requiring a customized treatment plan formulated for multiple tooth periodontal rehabilitation.

***Keywords*:** Periodontitis, Periodontal rehabilitation, Mucogingival surgery, Periodontal plastic surgery, Gingival recession, Root coverage, Periodontal regeneration

1.INTRODUCTION

In the recent decades, there has been a tremendous upgradation of the treatment strategies for multiple recession defects. Surgical procedures of the mucogingival complex aim at correcting defects in the morphology, position, or enhance the dental gingival junction, since defects in the morphology of the gingival and alveolar mucosa can accelerate the course of periodontal disease, Customized Planning in Multi-Tooth Periodontal Rehabilitation"or interfere with the successful outcome of periodontal treatment.[1,2]

The term ‘Periodontal Plastic Surgery’ was initially suggested by Miller in 1993. In modern periodontology it is becoming an acceptable and reliable treatment strategy which denotes “surgical procedures performed to prevent or correct anatomic, developmental, traumatic or disease-induced defects of the gingiva, alveolar mucosa or bone”. This definition includes different techniques for gingival augmentation, root coverage, soft-tissue augmentation at dental implants, crown-lengthening procedure, gingival preservation at teeth with ectopic eruption, removal of aberrant frena, prevention of ridge collapse and expansion of the edentulous ridge. [3-5]

The present case report is on such treatment procedure involving a customized treatment plan for multiple teeth's periodontal rehabilitation.

CASE REPORT

The primary complaint of a 23-year-old man who came to the Department of Periodontics and Implantology was widespread sensitivity in the lower front tooth region as well as the upper right and left tooth regions.

Patient gave a history of sensitivity in the upper right and left back tooth region for the past 3 months. Patient also complains of bleeding on brushing and food impaction of the upper left teeth region. Patient’s medical history was non contributory.

On extraoral examination, the patient’s face was grossly symmetrical, temporomandibular joint jaw movements were bilaterally synchronous, and regional lymphadenopathy was absent. On intra-oral examination, There was generalized diffuse gingival enlargement in both the jaws,with rolled out margins and blunt interdental papilla,loss of stippling, and change in the color and texture of the gingiva. Generalized Diffuse erythema was present on the marginal gingiva with a smooth shiny surface.

There was generalized bleeding on probing present in both jaws. Gingival recession was noted in 43,41,31,33. The probing depth was found to be more than 5 mm in most of the posterior tooth region. Furthermore, shallow vestibule of 3 mm in relation to 14,15,16

was recorded using University of North Carolina-15 (UNC-15) periodontal probe. (Figure 1)



Figure 1. Intraoral picture depicting gingival inflammation; (A): picture depicting gingival inflammation in the anterior region; (B): depicting gingival inflammation in the posterior region on the right side; and (C): picture depicting gingival inflammation in the posterior region on the left side.(E,F,G) Intra oral picture after scaling Orthopanthomography reveals depicted a generalized moderate to severe interdental alveolar bone loss. (Figure 5) The diagnosis of generalised periodontitis was made.



Figure 5,(A) Pre operative orthopantomogram showing bone loss in the maxilla and mandible (B)Post op orthopantomogram showing reduction of probing depth

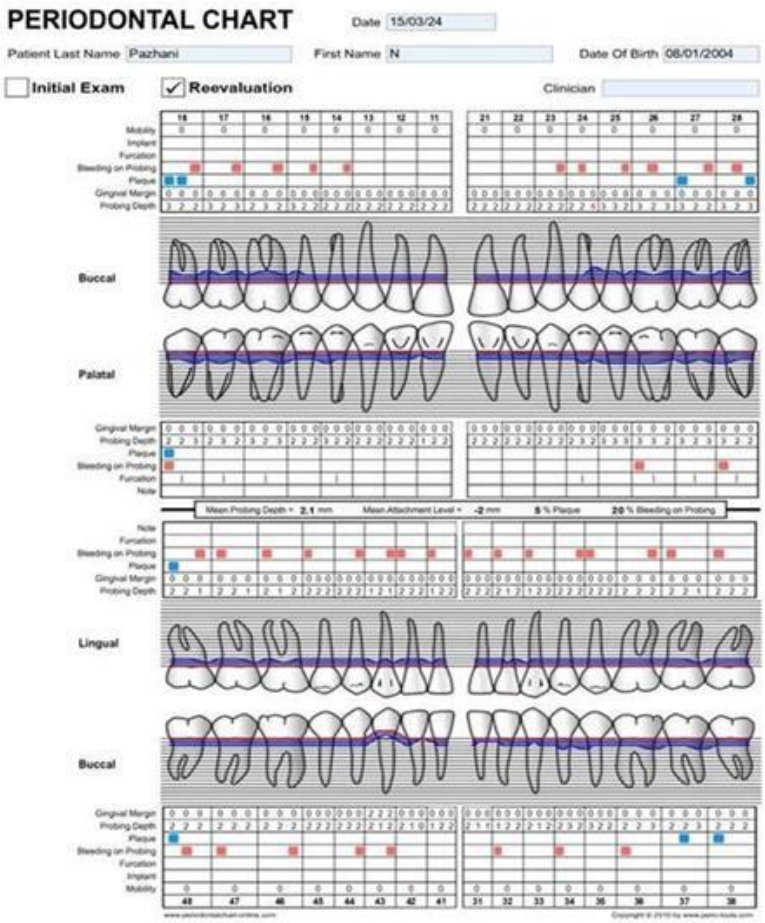


Figure 6 A. Preoperative periodontal chart

Initial periodontal therapy (IPT), and regenerative periodontal surgery as well as endodontic procedures was planned for the treatment. The patient received IPT including oral hygiene instructions, scaling and root planing using both hand and ultrasonic instruments. Patient was recalled after 2 weeks for flap surgery. Quadrant wise treatment was done i.e. The flap surgeries were performed in separate sessions for each quadrant. The patient was explained about the treatment procedure and written informed consent was obtained before the start of the treatment.

Right Maxillary arch

In the right maxillary arch, Periodontal flap surgery along with amniotic membrane graft and laser assisted vestibuloplasty was planned. Amniotic membrane graft was chosen for this quadrant, as it fulfills the mechanical concept of guided tissue regeneration demonstrating enhanced gingival wound healing properties and reduced scarring. [6]

Vestibuloplasty was planned as inadequate vestibular depth causes deficiency in width of keratinised gingiva which ultimately results in poor plaque control and difficulty in maintenance. Though several methods are used for achieving vestibuloplasty, in the present case lasers were used. Lasers offer several advantages such as minimal bleeding, faster healing, better visualisation, and minimal requirement of anaesthesia over conventional scalpel excision. [7]

Laser protective eyewear was worn by the operator, patient, and assistant. Surgical area was prepared with adequate anesthesia using 2% lignocaine hydrochloride containing adrenaline 1:200,000 was administered. The vestibuloplasty was then carried out to increase the width of the attached gingiva. Irrigated again with saline solution. Diode laser (Biolase™) emitting 940 nm wavelength was used for vestibuloplasty. It was adjusted at 1.5 watt power, pulsed contact mode, continuous pulse duration, and pulse interval of 1.00 ms. The incision was carried out at vestibule area using fibre optic laser tip of 300 µm diameter in a brushing stroke. The procedure was painless with minimal bleeding, reduced intraoperative time. (Fig 2D)

In the same sitting, crevicular incisions were made from mesial #13 to distal #17 using scalpel no.15. A full-thickness flap is opened by using the periosteal elevator until the mucobuccal fold is seen (Fig. 2A).

The operating area is irrigated with saline solution. The processed dehydrated amniotic membrane (Amnio-care dry amniotic membrane, Biocover Laboratories, Karnal, Haryana, India) was placed onto the exposed root surface and proximal bone.(Fig 2B) Upon placement, the processed dehydrated amnion allograft became hydrated and self-adhered to the exposed root and proximal bone, then the flap is coronally approximated (Fig 2C) and sutured with the sling suture (Fig. 2E).

The patient was prescribed systemic analgesics and antibiotics consisting of 500 mg of paracetamol every 6 h and 500 mg of amoxicillin every 12h for 5 days. The patient was instructed to continue his oral hygiene maintanance, except in the operated area, in which tooth brushing was done very gently using an extra soft bristle tooth brush after surgery and plaque control was maintained by means of gentle topical applications of chlorhexidine gluconate in saturated cotton swabs twice a day. The sutures were removed 1week after the surgery.

In the follow up period, there was an improvement in the gingival recession, increased width of keratinized gingiva and decrease in probing depth. (Figure 2 F,G,H,I,J)



Figure 2. Treatment in maxillary right quadrant; **(A).** Full-thickness flap raised in maxillary right quadrant; **(B).** Placement of amniotic membrane graft and coronally positioning of the flap**, (C).** Approximation of the flap **(D)** Laser assisted vestibuloplasty performed in relation to 14,15,26. **(E)** Sutures placed **(F)** Follow up of maxillary right quadrant at 2nd post-op week **(G, H)** At 1st post-op month (Reduction of the Probing depth and Increased width of keratinized tissue) **(I, J)** Follow up of maxillary right quadrant at 6month post op (Reduction of the Probing depth and Increased width of keratinized tissue)

Left Maxillary arch

In the second quadrant, a probing depth of 7 mm was noted in relation to 24,25. (Figure 5) Mucograft with periodontal flap surgery was planned in relation to 24,25. The Mucograft® is a three-dimensional collagen matrix that is an excellent soft-as well as hard tissue regeneration biomaterial. The mucograft's porosity allows for a greater penetration of mesenchymal cells into the transplanted area. Hence mucograft was the choice for grafting in relation to 24, and 25. [8]

Under local anesthesia, a full thickness mucoperiosteal flap was elevated using the periosteal elevator until the mucobuccal fold. Intrabony defect was seen in relation to 25 after complete debridement and removal of granulation tissue. Geistlich Mucograft® was placed on the connective tissue bed and was stabilized by periosteal sutures.Then the flap is positioned coronally and sutured with the sling suture (Fig.3).
Antibiotics and analgesics were prescribed for 5 days, and the patient was recalled after two week for suture removal. In the follow up period, there was an decrease in probing depth. (Fig3H)



Figure 3. Treatment for upper left arch; (A) flap elevation and visualization of the intrabony defects; (B) probing depth of 7 mm in relation to 24 and 25 (C) Mucograft used in the study; (D)Flap reflected debridment and granulation tissue removed (E) Placement of mucograft in the site (F) Stabilisation of mucograft with periosteal sutures; and (G) Suturing done (H)Follow up of maxillary left quadrant; at 2nd post-op week. At 6 month post op

Right mandibular arch

In the fourth quadrant, a gingival recession of 8 mm was noted in relation to 43. Mucograft with coronally advanced flap surgery was planned in relation to 42,43,33 the main aim is to increase the width of attached gingiva even when recession coverage is not fully obtained. Under local anesthesia, a full thickness mucoperiosteal flap and Geistlich Mucograft® was placed on the connective tissue bed and was stabilized by periosteal sutures. Then the flap is positioned coronally and sutured with the sling suture (Fig 4).
Antibiotics and analgesics were prescribed for 5 days, and the patient was recalled after a week for suture removal. In the follow up period, there was an improvement in the root coverage. (Fig 4E)



Figure 4. Treatment for mandibular right arch; (A) flap elevation and visualization of the intrabony defects; (B) Mucograft used in the study; (C) placement of Mucograft and stabilisation of Mucograft with periosteal sutures; and (D) Suturing done (E) Follow up of mandibular right arch quadrant; at 2nd post-op week; (B). At 6 month post op.

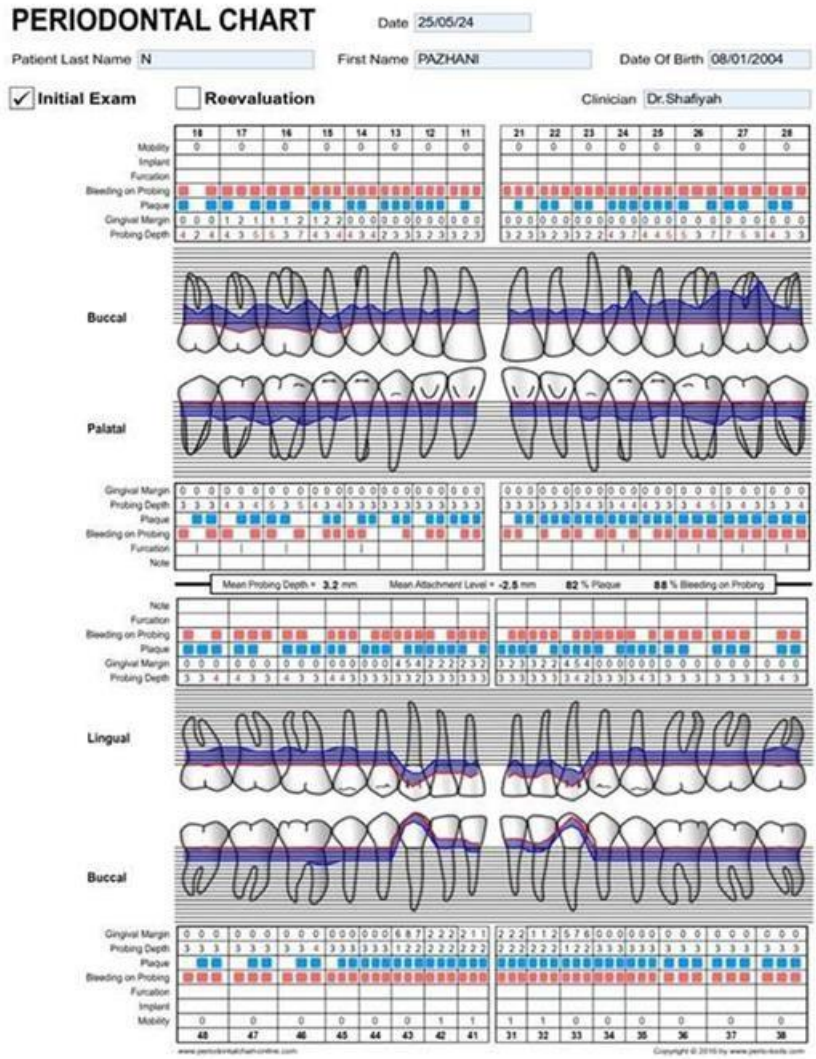


Figure 6B. Postoperative periodontal chart after 6 months

DISCUSSION

Periodontal disease is a most common chronic disease of the oral cavity and thereby is the major public health issue globally. [4,5] Periodontitis is a multifactorial disease with a complex interplay of host immune system and periodontal pathogens. [9] Though bacteria is considered as the prime cause of periodontal disease, the individual's inflammatory response as well as other modifying and predisposing factors ultimately determine the clinical presentation and outcome. [10] Disease progression is influenced by environmental and genetic factors that are unique to each person. [11] Thereby, this condition comprises a wide spectrum of conditions ranging from mild gingivitis to severe periodontitis. 12

The common features of periodontitis include gingival inflammation, clinical attachment loss, radiographic evidence of alveolar bone loss, sites with deep probing depths, mobility, bleeding upon probing and pathologic migration. [13,14] The goal of periodontitis treatment is to eliminate the causes and reduce modifiable risk factors. Initial non-surgical periodontal therapy primarily consists of scaling and root planing and oral hygiene maintenance.[15] Surgical management of periodontitis is preferred in cases unresponsive to non-surgical periodontal therapy and in severe cases.[16]

In the present case, mucogingival deformity caused by inadequate attached gingiva was seen in relation to the 14,15,16. Literature reveals that the presence of 2 mm or more of attached gingiva is considered necessary to maintain gingival health. [17] The presence of a thick keratinized gingival coating acts as a protective barrier against mastication pressures, as well as heat and chemical stressors. [8] Lang and Loe [17] reported that a significantly higher gingival index was noted for teeth with <2 mm of attached gingiva compared to those with at least 2 mm of attached gingiva.

To improve the mucogingival deformity caused by inadequate attached gingiva, laser assisted vestibuloplasty was performed in the present study. Vestibuloplasty is a mucogingival procedure that aims at surgical modification of gingiva-mucous membrane relationships including deepening of vestibular trough, altering position of frenulum or muscle attachments, and widening of zone of attached gingiva.[18] Soft tissue laser provides a predictable outcomes in periodontal therapy. [19]

The advantages of laser application are relatively bloodless surgery, minimal swelling, scarring and coagulation, no need for suturing, reduction in surgical time and less or no post surgical pain. Also, the laser instantly disinfects the surgical wound as well as allowing a non contact type of operative procedure and therefore no mechanical trauma to the tissue. [20,21] Nonetheless, reports have revealed a definite and clean wound was caused by laser and it eventually healed with secondary intention and no scar formation compared to scalpel incisions. This is may be due to the minimal degree of wound contraction following laser irradiation which occurs through induction and formation of smaller number of myofibroblasts and collagen. [22-24]

In the present case, Mucograft and amniotic membrane graft was used. The choice of graft was mainly based on the evidence based on the literature and the clinical presentation of a patient.

In recent years, Placenta-derived bioabsorbable membranes such as amnion and chorion have gained popularity in periodontal regeneration. Their unique application in periodontal treatment is mainly due to the presence of natural growth factors and collagen layers that are rich in type I, IV, V, and VI collagens.[25] It also contains laminin-5, plays a role in the cellular adhesion of gingival cells. Amnion membrane is a thin, tough, and transparent membrane that closely mimics the basement membrane of human oral mucosa with self-adherence and good aesthetic properties and appears to be smooth, shiny, and easily manageable. [26]

Literature reveals that Amnion membrane is an excellent choice as a graft in the treatment of gingival recession [25,32], furcation defects [26], and intrabony defects [27,28]. Especially, favourable results in covering the gingival recessions and in increasing the thickness of the attached gingiva [29,30]. Despite these advantages and promising role in periodontal regeneration, it has few limitations including operator skill requirement, risk of contamination, fragility, and technique-sensitivity.[31]

Another graft material used in the present case was Mucograft. It is a resorbable commercially available three-dimensional (3D) matrix that is designed specifically for soft tissue regeneration in the oral cavity. [33] It is a matrix of pure type I and III porcine collagen. [34]

Rokn et al.[34] conducted a randomized control trial to compare Mucograft® and FGG in augmenting the keratinized tissue around the teeth. It was found that the Mucograft® was superior when compared to FGG in terms of gain in keratinized tissue, less pain, less surgical chair time, and better aesthetics. Navya et al [8] reported that the Mucograft® was a feasible treatment option for soft-tissue augmentation. Bevilacqua et al [35] reported that Mucograft is an effective grafting material for increasing the thickness of the peri-implant mucosa and in the gaining of keratinized gingiva.

In the present case, at the second month follow up, we noted excellent results in root coverage, decrease in probing depth and increase in width of keratinized tissue. A customized treatment plan for multiple teeth's periodontal rehabilitation was formulated for this case. This custom made plan has led to better improvement in the periodontal status of the patient.

CONCLUSION

A well-structured treatment plan is essential to ensure optimal outcomes, particularly in the periodontal rehabilitation of multiple teeth. In the present case the patient demonstrated excellent outcomes, including effective root coverage, reduced probing depths, and an increase in the width of keratinized tissue.

DECLARATIONS

Conflicts of interest

The authors declare no conflict of interest.

Funding

This research received no external funding.

Informed Consent Statement

The patient provided an informed consent statement.

Ethical Statement

This case report was conducted in accordance with institutional ethical standards. Written informed consent was obtained from the patient for the publication of this case and accompanying clinical data.

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COMPARATIVE EVALUATION OF DIFFERENT PROCEDURES FOR ANALYZING RESIDUAL ALVEOLAR RIDGE WIDTH BEFORE DENTAL IMPLANT PLACEMENT: A CLINICAL STUDY

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ABSTRACT

Background: Accurate assessment of the residual alveolar ridge width is critical for successful dental implant placement. Multiple techniques exist to measure this parameter, ranging from radiographic to clinical and non-invasive imaging methods.

Objectives: To compare the reliability, accuracy, and clinical applicability of six different techniques—direct surgical measurement (gold standard), CBCT, ridge mapping, ultrasound, digital caliper, and occlusal radiography—for evaluating residual alveolar ridge width prior to dental implant placement.

Materials and Methods: Sixty patients (35 females, 25 males; mean age 52.7±11.3 years) with 85 implant sites were evaluated using all six techniques. Ridge width measurements were recorded at three levels (crestal, 3 mm, and 6 mm apical) using each method. Direct surgical measurement during implant placement served as the gold standard.

Results: Mean ridge width by direct surgical measurement was 4.20±1.58 mm. Ridge mapping (4.57±1.63 mm), CBCT (4.62±1.67 mm), and digital caliper (4.25±1.60 mm) showed no significant difference from the gold standard ($p > 0.05$). Occlusal radiography significantly overestimated ridge width (5.10±1.80 mm, $p<0.001$). Ridge mapping showed highest accuracy (95%) and correlation (r=0.95), followed by digital caliper (94%, r=0.94) and CBCT (92%, r=0.92). Ultrasound showed moderate accuracy (89%) and correlation (r=0.89), while occlusal radiography was least accurate (85%, r=0.82). Intra-examiner and inter-examiner reliability were excellent across all techniques (ICC range: 0.85–0.98).

Conclusion: Ridge mapping and CBCT are reliable, accurate, and clinically effective non-invasive alternatives for evaluating alveolar ridge width before implant placement. Ridge mapping offers a radiation-free, cost-effective chairside solution, while CBCT provides comprehensive anatomical details with higher cost and radiation exposure. Ultrasound is promising but requires further refinement to match the accuracy of established methods. Ridge mapping and CBCT are reliable, accurate, and clinically effective non-invasive alternatives for evaluating alveolar ridge width before implant placement. Ridge mapping offers a radiation-free, cost-effective chairside solution, while CBCT provides comprehensive anatomical details with higher cost and radiation exposure. Ultrasound is promising but requires further refinement to match the accuracy of established methods.

Keywords**:** Alveolar ridge width, dental implants, ridge mapping, CBCT**.**

INTRODUCTION

Dental implants have transformed rehabilitation of edentulous patients giving them both aesthetic and functionality better than the conventional methods of replacing missing teeth 1,2. Successful long-term survival of a dental implant is greatly determined by its proper treatment planning where proper evaluation of available bone dimensions is very imperative [3,4]. Low bone volume specially ridge width is one of the usual difficulties in implant dentistry which can require bone grafting techniques or other treatment modalities [5,6].

A major resorption of alveolar ridge will take place after extraction of tooth such that the horizontal loss of bone is much faster as well as widespread compared with the vertical resorption [7,8]. Research has proven that buccolingual dimension may drop up to 50 percent in a year after extraction process, two thirds of the degradation being in the first three months [9,10]. Such dimensional alteration underlines the relevance of an adequate ridge width estimation before inserting the implant [11,12].

Several methods have been adopted to measure the width of the alveolar ridge and they all bear their own merits and demerits [13]. The standard two dimensional radiograph makes it difficult to capture the information about the buccolingual dimensions though they are useful in assessing the heights [14]. Imaging technology including CBCT provides a complete three-dimensional image but is based on radiation and more expensive [15]. The clinical tools such as ridge mapping, or direct caliper measure result in immediate results, but are invasive and dependent on an operator.

New technologies have brought new methods like ultrasound imaging as well as digital integration of workflow and may provide non-invasive solutions with lower intensities of radiation [1]. Although various measurement methods are available, no sufficient agreements seem to exist on the relative accuracy, reliability, and clinical relevance of the methods [2].

This paper intends to critically discuss the six various methods of measuring residual alveolar ridge width prior to surgical placement of dental implants; CBCT, ridge mapping, direct surgical measurement, ultrasound, digital caliper, and occlusal radiography. By evaluating these techniques in a variety of parameters such as accuracy, radiation exposures, cost, and invasiveness, this study aims to present evidence-based implications to clinical practice.

Materials and Methods

Patient selection and Study Design

Sixty patients (35 females and 25 males) undergoing treatment and having age range 2874 years, in which dental implants have to be placed, were enrolled in the study. Inclusion criteria included patients 18 years or above, at least one edentulous site, and there should be implant placement, the extraction site should be healed with a minimum of 4 months and patient should have good oral hygiene. Systemic conditions that made implant surgery unfeasible, radiotherapy to the head and neck, uncontrolled diabetes, women who were pregnant, and who were unable to adhere to the study protocol were the exclusion criteria.

Measurement Techniques

Six different techniques were employed to measure the buccolingual width of the alveolar ridge at each implant site. Cone Beam Computed Tomography (CBCT) scans were obtained using a high-resolution scanner, and measurements were performed using dedicated software by an experienced radiologist blinded to the clinical data. Ridge mapping involved the fabrication of a vacuum-formed stent with reference holes, through which a bone caliper was used under local anesthesia to record measurements at three levels: crestal, 3 mm apical, and 6 mm apical to the crest. Direct surgical measurements were taken during implant surgery following flap elevation using a digital caliper at the same three levels, serving as the gold standard for comparison. A high-frequency ultrasound device with a 15 MHz linear transducer was employed to obtain cross-sectional images of the alveolar ridge, with measurements recorded using integrated software. A modified digital caliper with pointed tips was used under local anesthesia to measure the ridge width trans-mucosally at the specified levels. Occlusal radiographs were obtained using a standardized paralleling technique with a film holder and grid, and measurements were performed using calibrated digital software. All measurements were carried out independently by two calibrated examiners, and the mean values were used for analysis. To assess intra-examiner reliability, 20% of the measurements were repeated after a two-week interval.

Statistical Analysis

Sample size calculation was performed using appropriate statistical software, determining that a minimum of 54 implant sites was needed to detect a mean difference of 0.5 mm between techniques with 80% power at a 5% significance level. Statistical analysis was conducted using SPSS software. Descriptive statistics including mean, standard deviation, and range were computed for all measurements. The Shapiro-Wilk test was used to assess the normality of the data. One-way analysis of variance (ANOVA) with post-hoc Tukey's test was employed to compare mean values across measurement techniques. Pearson correlation coefficients were calculated to determine the relationship between each method and the gold standard.

Bland-Altman analysis was used to assess agreement between methods, with 95% limits of agreement calculated. Intra- and inter-examiner reliability were evaluated using intraclass correlation coefficients (ICC). A p-value of less than 0.05 was considered statistically significant for all analyses.

The study included 60 patients (35 females, 25 males) with a mean age of 52.7±11.3 years. A total of 85 implant sites were evaluated, distributed as follows: maxillary anterior (n=18), maxillary posterior (n=24), mandibular anterior (n=15), and mandibular posterior (n=28).

The mean healing time post-extraction was 8.3±2.7 months (range: 4–18 months).

3. RESULTS

The mean alveolar ridge width measurements obtained bydifferent techniques are presented in **Table 1** and **Figure 1**. Direct surgical measurement, considered the gold standard, yielded a mean width of 4.20±1.58 mm. The highest mean value was recorded with occlusal radiography (5.10±1.80 mm), followed by CBCT (4.62±1.67 mm), ridge mapping (4.57±1.63 mm), ultrasound (4.50±0.93 mm), and digital caliper (4.25±1.60 mm).

Table 1. Mean alveolar ridge width measurements by different techniques

Technique	Mean ± SD (mm)	Range (mm)	Mean Difference from Direct Measurement (mm)	p-value
Direct Surgical Measurement	4.20 ± 1.58	2.00 - 9.00	-	-
Ridge Mapping	4.57 ± 1.63	2.00 - 9.00	0.37 ± 0.15	0.381
CBCT	4.62 ± 1.67	1.80 - 9.30	0.42 ± 0.18	0.318
Ultrasound	4.50 ± 0.93	2.50 - 7.80	0.30 ± 0.21	0.452
Digital Caliper	4.25 ± 1.60	2.10 - 8.90	0.05 ± 0.12	0.842
Occlusal Radiography	5.10 ± 1.80	2.30 - 9.50	0.90 ± 0.22	<0.001*

*Statistically significant difference (p<0.05)

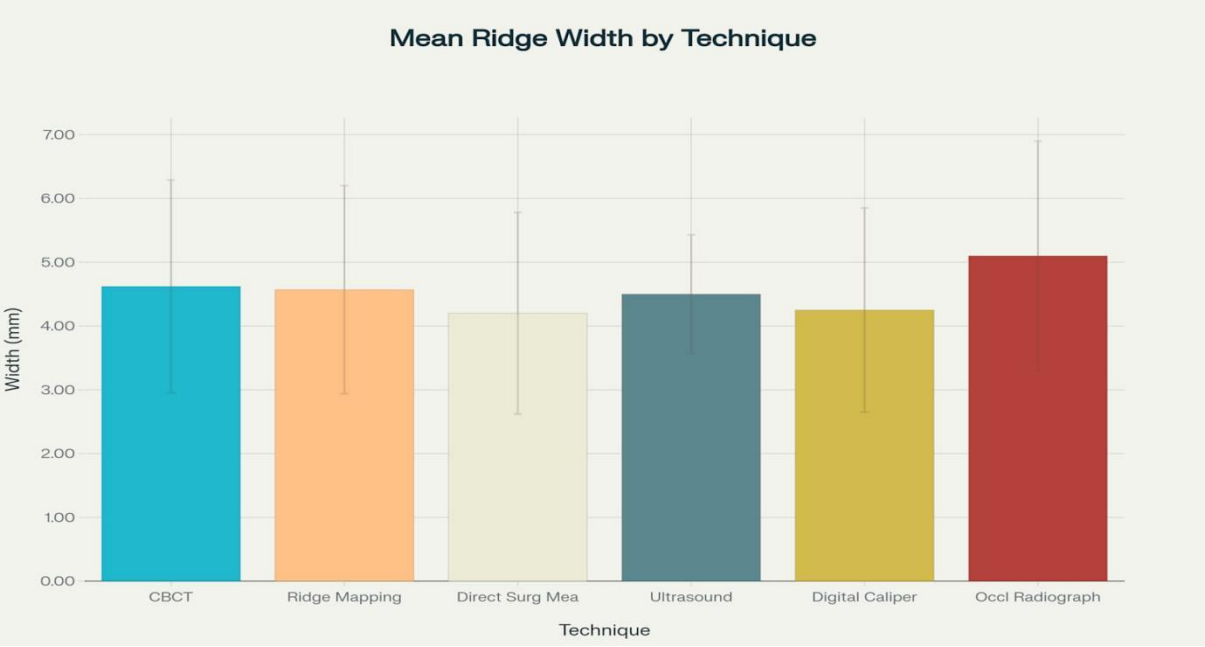


Figure 1. Mean ridge width measurements (mm) by different measurement techniques with standard deviations

Figure 1 illustrates the mean ridge width measurements with standard deviations for each technique. One-way ANOVA showed statistically significant differences between the techniques (F=3.87, p=0.002). Post-hoc analysis indicated that occlusal radiography values were significantly higher than direct surgical measurements (mean difference: 0.90±0.22 mm, p<0.001). No statistically significant differences were found between direct surgical measurement and ridge mapping, digital caliper, or CBCT.

The accuracy of each technique compared to the gold standard is summarized in **Table 2** and visually represented in **Figure 2**. Ridge mapping demonstrated the highest accuracy (95%), followed by digital caliper (94%), CBCT (92%), ultrasound (89%), and occlusal radiography (85%).

Pearson correlation analysis revealed strong positive correlations between direct surgical measurement and ridge

mapping (r=0.95), digital caliper (r=0.94), and CBCT (r=0.92). Moderate correlations were observed with ultrasound (r=0.89) and occlusal radiography (r=0.82).

Bland-Altman analysis demonstrated the narrowest 95% limits of agreement for ridge mapping (-0.46 to 1.20 mm), followed by digital caliper (-0.52 to 0.62 mm), CBCT (-0.58 to 1.42 mm), ultrasound (-0.75 to 1.35 mm), and occlusal radiography (-0.65 to 2.45 mm).

Intra-examiner reliability was excellent for all techniques (ICC range: 0.92–0.98). Inter-examiner reliability was highest for CBCT (ICC=0.96), followed by direct surgical measurement (ICC=0.95), ridge mapping (ICC=0.93), digital caliper (ICC=0.91), ultrasound (ICC=0.88), and occlusal radiography (ICC=0.85).

Table 2. Accuracy and correlation of measurement techniques compared to direct surgical measurement

Technique	Accuracy (%)	Correlation Coefficient (r)	p-value	95% Limits of Agreement (mm)
Ridge Mapping	95	0.95	<0.001	-0.46 to 1.20
CBCT	92	0.92	<0.001	-0.58 to 1.42
Ultrasound	89	0.89	<0.001	-0.75 to 1.35
Digital Caliper	94	0.94	<0.001	-0.52 to 0.62
Occlusal Radiography	85	0.82	<0.001	-0.65 to 2.45

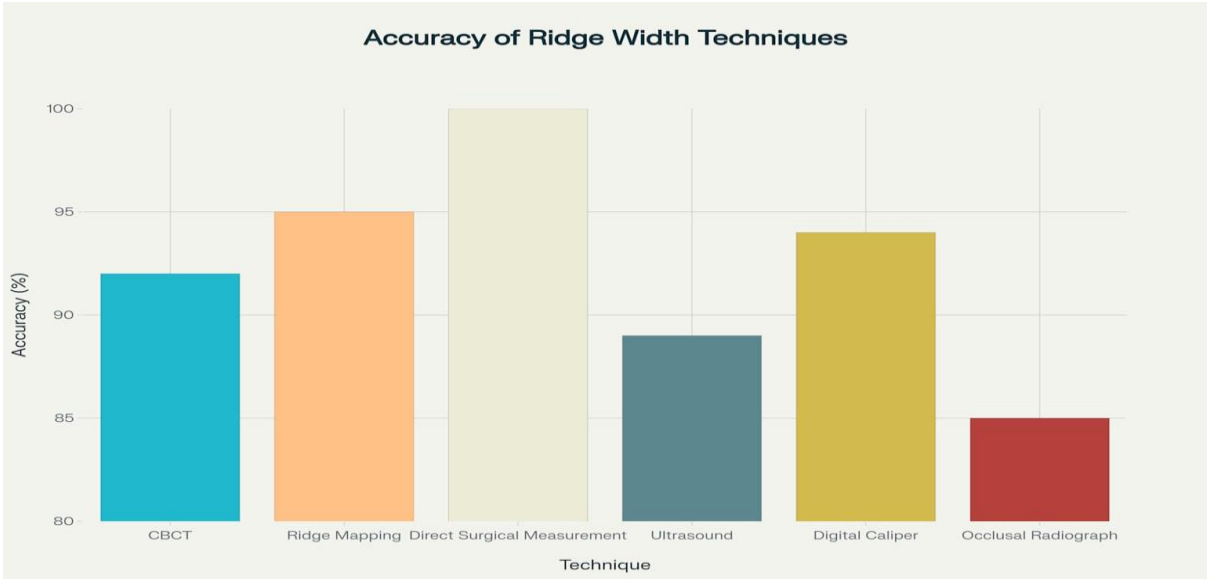


Figure 2. Accuracy of different ridge width measurement techniques compared to direct surgical measurement (gold standard)

A comprehensive evaluation considering accuracy, radiation exposure, cost, invasiveness, and chairside applicability is presented in **Table 3** and **Figure 3**. Although direct surgical measurement provided the highest accuracy, it was also the most invasive. Ridge mapping and digital caliper showed excellent accuracy with moderate invasiveness and low cost. CBCT offered good accuracy but involved radiation exposure and higher cost. Ultrasound was a promising non-invasive alternative with no radiation exposure but relatively higher cost.

Table 3. Comprehensive evaluation of measurement techniques

Technique	Accuracy (%)	Radiation Exposure	Relative Cost	Invasiveness	Chairside Availability
Direct Surgical Measurement	100	None	Moderate	High	Yes
Ridge Mapping	95	None	Low	Moderate	Yes
CBCT	92	High	High	None	No
Ultrasound	89	None	Moderate-High	None	Yes
Digital Caliper	94	None	Low	Moderate	Yes
Occlusal Radiography	85	Moderate	Low-Moderate	None	Yes

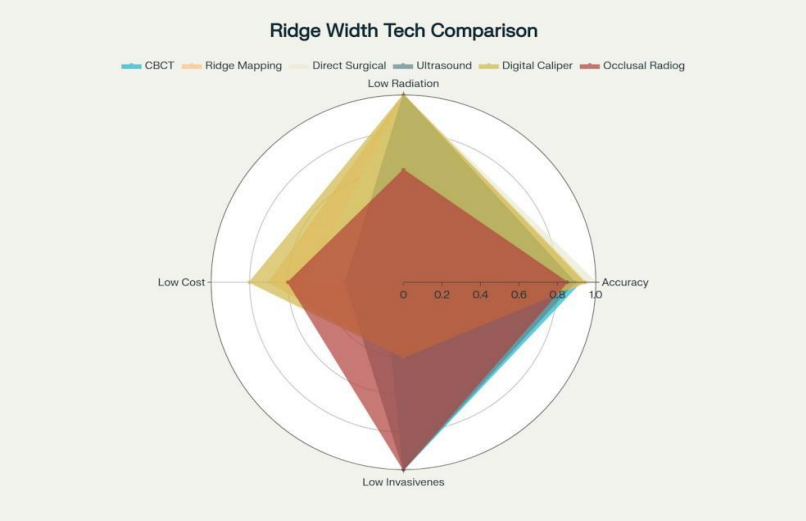


Figure 3. Comparison of key factors across different ridge width measurement techniques

DISCUSSION

The correct evaluation of the width of the alveolar ridge is a keystone in the process of dental treatment planning of implants [3,4]. The dominant feature of this research was a thorough comparison of six procedures to analyze the residual alveolar ridge width, and it offered great results in relation to the comparative value of these procedures [5,6].

We reported that the values of the ridge mapping, digital caliper and CBCT were not statistically significantly different compared with the value of the direct surgical measurement that was used as a gold standard [7-9]. This is in line with the earlier study by Chugh et. al., who had no remarkable reduction in the interventions between ridge mapping, CBCT and direct measurement [3]. In the same manner, Castro-Ruiz et al. reported a high concordance rating (ICC 0.82) between ridge mapping and CBCT measurements [6].

The most accurate (95%) and highly correlated (r= 0.95) test (non-invasive) with direct surgical measurement was the ridge mapping [10]. This is backed by the study of Chan et al who reported that ridge mapping is an effective tool in the measurement of alveolar ridge width because of its accuracy, non-costly nature, quick turnaround time and it is not associated with any form of radiations [11]. Ridge mapping presented the minor overestimation (mean difference: 0.37 0.15 mm) which could be explained by the effect of the soft tissues compression during the measurement, proposed by Bohner et al. [12].

CBCT displayed high accuracy (92%) and excellent correlation (r=0.92) with direct measurement as it was seen that the results were very similar to Taheri et al., who reported that the mean difference between CBCT and direct surgical measurements was not statistically significant [13]. But, in our Bland-Altman plots, the limits of agreement of CBCT (-0.58 to 1.42 mm) were observed to be higher than ridge mapping, which means that, CBCT has more variability in measurements. Such variability could be attributable to voxel size, beam hardening artifacts, and errors that could be caused by software, as mentioned by Choi et al. [14].

Ultrasonography The ultrasound method is a comparatively newer method of ridge width measurement, in our study, it revealed moderate accurateness (89%) [15]. It is in line with the recent investigation by Chan et al. who has stated that high-resolved ultrasound was promising as a crestal bone ridge width assessment tool [11]. Non-invasiveness and the fact that it is radiation-free makes ultrasound an appealing alternative, but right now, not of the same accuracy as ridge mapping and CBCT [16].

Occlusal recordings had an 85 percent accuracy and greatly exaggerated the width of the ridge (mean difference: 0.90+/-0.22 mm, $p<0.001$) [17]. This may be inaccurate due to the aspect of magnification and distortion in two dimensional radiographic procedures [12].

There is more to the accuracy as some practical considerations also play a factor in the application of various methods of measurement in the clinical setting. We looked at radiation exposure, cost, invasiveness, and chairside availability to come up with a total perspective of each of the methods [5].CBCT it provides a detailed plan of treatment as it provides a thorough three dimensional image of alveolar ridge and other anatomical structures around. Nevertheless, its application implies radiation exposure, although at less high doses than conventional CT, and increased cost compared to clinical ones [18].

Both ridge mapping and use of digital caliper are instant at chairside and have high accuracies and low costs. Such methods demand the use of a local anesthetic and are somehow invasive, thereby possible to produce discomfort to the patient. However, they are very useful in everyday clinical practice due to simplicity, portability, and reliability, particularly when there is a need to avoid radiation [10,11].

Ultrasound is another non- invasive technology that is promising in the sense that it does not expose patients to radiations and gives a real time picture. Although in our study it showed moderate accuracy, future technological changes can make it more precise and clinically applicable. They also mentioned that as high-resolution ultrasound had the potential of becoming a chair-side screening tool in determining the ridge width combined with other forms of diagnosis [16].

The research possesses a number of strengths as it is prospective, has evaluated a vast range of techniques, and its statistics are sound [6]. The introduction of new techniques like ultrasound besides the traditional ones is also of great importance as it offers an insight into the new technologies [15].

Nevertheless, some restrictions are to be mentioned [6]. One, the research was done in one center, which may have restrained the applicability of results [7]. Second, measurements were carried out at three preselected levels and this might fail to reflect the full morphology of the alveolar ridge [8]. Third, the effect of the soft tissue thickness and ridge morphology on accuracy during measurements was not analyzed per se [9]. Lastly, the research concerned linear measures of ridge width, excluding three-dimensional volumetric analysis [13].

CONCLUSION

The present study points out ridge mapping (95% accurate) and CBCT (92%) as the best alternatives of non-invasive techniques to calculate alveolar ridge width. The choice of technique ought to be concerned with a balance between accuracy, invasiveness, cost and clinical setting. Improvement of the ultrasound accuracy and research on existing relationships between measurement precision and the success of the implants should be conducted in the future.

DECLARATIONS

Ethics Approval and Consent to Participate

Ethical approval was obtained from the Institutional Ethics Committee.

Consent for Publication

The patient provided written informed consent for the publication of this case report, including clinical images and radiographic findings.

Competing Interests

The authors declare that they have no competing interests related to this study.

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ORIGINAL RESEARCH

A CEPHALOMETRIC STUDY OF PARENTAL CRANIOFACIAL PARAMETERS TO DETERMINE THE PREDISPOSITION TO OROFACIAL CLEFTING

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ABSTRACT

AIM To determine the craniofacial morphological features of parents of children with oro-facial clefting and to correlate the findings with those of other investigators and to determine if the predisposition of the anomaly is related to craniofacial morphology.

Methods and Materials: This study was conducted in the Department of Oral and Maxillofacial surgery, A.B. Shetty Memorial Institute of Dental Sciences, Deralakatte, Mangalore, Karnataka , India with the intention of studying the craniofacial morphology of parents of cleft children and to compare them with that of the control group. The study was conducted from 2001 to 2003.

Results: Multivariate discriminant analysis was applied to the data. When fathers of cleft children who were not from the parental pair group were compared to fathers of children without orofacial clefting (control), they were found to have (i) significant reduction in the symphysis area (p=0.025)(ii)Angle SNB was more acute (p=O.045, significant)(iii)Had a greatly reduced mandibular area (p=0.000 vhs)(iv)Reduced maxillary area (p=0.001 vhs)(v)An increased SN length hs) (vi)Cranial base angle was more acute (p=0.002hs).

Conclusion: Conducting such a study and understanding these parameters could be valuable in predicting susceptibility to cleft formation. It may also aid in uncovering clues about the development of cleft palate and cleft lip conditions. This could potentially serve as part of a range of tests, including assessments on lateral cephalograms for prospective parents concerned about the likelihood of clefts in their children, enabling healthcare professionals to offer informed guidance.

***Keywords**: Cephalometrics , craniofacial , hereditary , oral clefts*

INTRODUCTION

Cleft lip/palate is one of the most common congenital malformations of the face. It is prevalent among all races irrespective of sex. The incidence of cleft lip and palate in India is one in eight hundred live births 1989.[1] Orofacial clefts are caused by interactions between genetic and environmental factors.[2] It is understood that genetic -factors create a 'Susceptibility' for clefts when environmental factors

(triggers) interact with a genetically susceptible genotype and a cleft develops. [2,3]

The theory of etiological heterogeneity has now taken precedence, suggesting that genetic factors in the occurrence of oral clefts vary widely. In some cases, genetic influence is minimal; in others, it is strongly associated with one parent, and in certain situations, it is equally shared when both parents happen to have similar levels of predisposing factors. Based on epidemiological evidence, different inheritance patterns for craniofacial morphology have been proposed.

Research on hereditary impacts in orofacial clefting reveals that relatives without clefts often possess unique facial traits that set them apart from the general population. It is understood from these studies that such features reflect the expression of genetic susceptibility to clefting. Many studies have been done on facial morphology of parents of children with clefts of the lip and palate[9], the dentition of parents[.8] Dermatoglyphics in parents and on craniofacial parameters with the help of lateral and PA skull radiographs.

In current thinking, the multi-factorial threshold (MFT) model has been replaced by the theory of etiological heterogeneity.[13] This theory proposes that genetic factors in the development of oral clefts vary: in some cases, genetic influence is minimal; in others, it is primarily attributed to one parent; and in situations where both parents share similar levels of predisposition, the genetic influence is roughly equal.

MATERIALS AND METHODS

This comparative cephalometric study was conducted in the Department of Oral and Maxillofacial Surgery, A.B. Shetty Memorial Institute of Dental Sciences, Deralakatte, Mangalore, Karnataka, India, from 2001 to 2003. Ethical approval was obtained prior to the commencement of the study.

Sample Selection:

A total of 90 participants were included:

Study Group (n=60):

- Parental pairs of cleft children (n=15 pairs)
- Fathers only (n=15)
- Mothers only (n=15)
- Control Group (n=30):
- Fathers of non-cleft children (n=15)
- Mothers of non-cleft children (n=15)

All participants in the study group were parents of children with unilateral cleft lip and/or palate. Subjects with prior orthodontic or maxillofacial surgical history were excluded. The control group was randomly selected from the outpatient department and included parents of children without any congenital, genetic, endocrinal, or skeletal anomalies.

Radiographic Protocol:

Standardized lateral cephalometric radiographs were taken using a Planmeca panoramic radiographic unit. A total of 90 lateral cephalograms were obtained (60 from the study group, 30 from controls).

Cephalometric Analysis:

Parameters Analyzed: 20 cephalometric variables (10 midface, 10 lower face) were recorded for each subject.

Measurement Technique: Cephalometric landmarks were digitized using AutoCAD R14 software to compute areas and perimeters.

RESULTS

The morphological evaluation of cephalometry proved that there were considerable differences between study group (parents of children with orofacial clefting) and control group (parents of children without cleft anomalies) in certain parameters concerning the mandibular region and the mid-face. In the group of fathers of cleft-affected children, there was a statistically significant decrease in the symphysis area ($p = 0.047$) and the total mandibular area ($p = 0.000$) that referred to a smaller design of the mandibular structure in the cleft-prone parent group. Compared to the control group, the SNB angle was sharper in the study group ($p = 0.045$), and it indicated a comparatively retruded mandible. Also of greater significance was the increase in the sella-nasion (SN) length and sharper cranial base angle ($p = 0.002$), which was further distinct to their control counterparts. The greatest differences among mothers differed in total mandibular length that was significantly higher in the mothers of cleft children as compared to the controls ($p = 0.000$). These individuals showed themselves much taller in the anterior face ($p = 0.000$), more obtuse cranial base angle ($p = 0.002$) and longer anterior cranial base (SN length $p = 0.000$ as well).

These characteristics indicate that the craniofacial morphology of mothers could be larger in vertical features and protuberance towards the anteriors in the cleft affiliated cases.

Further significant differences were also observed when parental pair fathers contrasted against control fathers. These comprised the smaller area of symphysis (p = 0.047), a shorter length of palate (p = 0.003) and a much smaller area of maxillary region (p = 0.027), with a longer SN measures (p = 0.018) and a more acute cranial base angle (p = 0.016). These differences support the hypothesis that smaller midface and mandibular measurements can be phenotypic predictors of susceptibility to clefts in parental pair mothers their total mandibular length was longer (p = 0.000), their anterior facial height was greater (p = 0.005), their SN length was longer (p = 0.000) and the angle components of their cranial base were sharper (p = 0.000). It is also important to note that the length of the clivus (S-Ba) was significantly longer among these mothers (p = 0.000), and this fact can be related to the structural defects of the cranial base that also leads to a risk of developing clefts. Among the five powerful discriminants between cleft and non cleft groups included in the multivariate analysis by use of the Mahalanobis distance was the five cephalometric variables in fathers (total mandibular area, cranial base angle, palatal length, SN length and symphysis area). In mothers the total mandibular length and cranial base angle were the most important. These discriminant function analysis concluded the existence of sexually dimorphic patterns in cephalometric features relative to cleft risk. (Table 1-5)

Statistical Analysis

Student's unpaired t-test was used for pairwise group comparison. Multivariate discriminant analysis (Mahalanobis distance) was applied to determine morphological features predictive of cleft predisposition. The statistical analysis used for the results obtained were Student's unpaired t test' and multivariate discriminant analysis using Mahalanobis distance.[9]

RESULTS

The morphological evaluation of cephalometry proved that there were considerable differences between study group (parents of children with orofacial clefting) and control group (parents of children without cleft anomalies) in certain parameters concerning the mandibular region and the mid-face. In the group of fathers of cleft-affected children, there was a statistically significant decrease in the symphysis area (p = 0.047) and the total mandibular area (p = 0.000) that referred to a smaller design of the mandibular structure in the cleft-prone parent group. Compared to the control group, the SNB angle was sharper in the study group (p = 0.045), and it indicated a comparatively retruded mandible. Also of greater significance was the increase in the sella-nasion (SN) length and sharper cranial base angle (p = 0.002), which was further distinct to their control counterparts. The greatest differences among mothers differed in total mandibular length that was significantly higher in the mothers of cleft children as compared to the controls (p = 0.000). These individuals showed themselves much taller in the anterior face (p = 0.000), more obtuse cranial base angle (p = 0.002) and longer anterior cranial base (SN length p = 0.000 as well). These characteristics indicate that the craniofacial morphology of mothers could be larger in vertical features and protuberance towards the anteriors in the cleft affiliated cases. Further significant differences were also observed when parental pair fathers contrasted against control fathers. These comprised the smaller area of symphysis (p = 0.047), a shorter length of palate (p = 0.003) and a much smaller area of maxillary region (p = 0.027), with a longer SN measures (p = 0.018) and a more acute cranial base angle (p = 0.016). These differences support the hypothesis that smaller midface and mandibular measurements can be phenotypic predictors of susceptibility to clefts in parental pair mothers their total mandibular length was longer (p = 0.000), their anterior facial height was greater (p = 0.005), their SN length was longer (p = 0.000) and the angle components of their cranial base were sharper (p = 0.000). It is also important to note that the length of the clivus (S-Ba) was significantly longer among these mothers (p = 0.000), and this fact can be related to the structural defects of the cranial base that also leads to a risk of developing clefts. Among the five powerful discriminants between cleft and non cleft groups included in the multivariate analysis by use of the Mahalanobis distance was the five cephalometric variables in fathers (total mandibular area, cranial base angle, palatal length, SN length and symphysis area). In mothers the total mandibular length and cranial base angle were the most important. These discriminant function analysis the existence of sexually dimorphic patterns in cephalometric features relative to cleft risk(Table 1-5).

Table 1. Parameters Used for Comparison

Lower Face Parameters	Mid-Face Parameters
1. Ramus Height	1. ANS–PNS (Length of Maxilla)
2. Gonial Angle	2. Area of Maxilla
3. Body Length	3. Angle SNA
4. Symphysis Area	4. N–A (Nasion to Point A)
5. Total Mandibular Area	5. AFH (Anterior Facial Height)
6. Total Mandibular Length	6. PFH (Posterior Facial Height)
7. SNB Angle	7. Ratio PFH:AFH
8. Pt. B	8. UFH (Upper Facial Height)
9. Pog	9. LFH (Lower Facial Height)
10. MM Angle	10. Ratio UFH:LFH

Table 2. Variables and Criteria Used for Methodology

Variable Type	Criteria for Significance
Linear Distance Measurements	> 2 mm difference in the means of statistically significant measurements
Angular Measurements	> 2 degrees difference in the means of statistically significant measurements
Area Measurements	> 10% difference in the means of statistically significant measurements

Table 3. Results (t-test for Equality of Means) Mandibular Parameters Comparison

Sex	Parameter	t-value	p-value
Fathers	Ramus height	0.302	0.765
	Gonial angle	1.042	0.306
	Body length	-1.050	0.303
	Symphysis area	-2.080	0.047
	Total Mandibular Area	-6.715	0.000
	Total Mandibular Length	0.057	0.955
	SNB angle	0.838	0.409
	N–B	1.441	0.161

	N–Pog	0.979	0.336
	MM angle	-0.262	0.795
Mothers	Ramus height	-0.234	0.817
	Gonial angle	0.296	0.769
	Body length	1.428	0.164
	Symphysis area	0.507	0.955
	Total Mandibular Area	1.059	0.298
	Total Mandibular Length	6.880	0.000
	SNB angle	0.950	0.350
	N–B	0.151	0.881
	N–Pog	-0.377	0.709
	MM angle	0.443	0.661

Table 4. Mid-Face Parameters and Discriminant Function Analysis Mid-Face Parameters Comparison – t-test

Sex	**Parameter**	**t-value**	**p-value**
Fathers	ANS–PNS	3.204	0.003
	Area of Maxilla	2.342	0.027
	Angle SNA	0.314	0.756
	N–A	1.056	0.300
	AFH	0.108	0.915
	PFH	1.134	0.266
	PFH:AFH	0.787	0.438
	UFH	-1.322	0.197
	LFH	0.577	0.569
	UFH:LFH	-1.127	0.269
Mothers	ANS–PNS	-0.364	0.719
	Area of Maxilla	-1.766	0.088
	Angle SNA	0.606	0.550
	N–A	0.067	0.947
	AFH	3.014	0.005
	PFH	0.212	0.833
	PFH:AFH	0.072	0.943

	UFH	-1.186	0.246
	LFH	1.021	0.316
	UFH:LFH	-1.407	0.170

Standardized Canonical Discriminant Function Coefficients (Mahalanobis Distance)

Sex	Discriminant Variable	Function Coefficient
Fathers	Symphysis Area	0.427
	Total Mandibular Area	0.844
	ANS–PNS	0.538
	Cranial Base Angle	0.742
	SN Length	0.520
Mothers	Cranial Base Angle	0.518
	Total Mandibular Length	0.874

Table 5. Summary of Previous Parental Cephalometric Studies in Orofacial Clefting

Study / Author	Experimental Group (Total/Male/Female)	Comparison Group	Population	OFC Subtypes	Cephalogram Type
Coccaro et al. (1972)	40 / 20 / 20	40	USA	CLP	Lateral
Kurisu et al. (1974)	347 / 141 / 206	246	USA	CL(P), CP	Lateral, PA
Shibosaki et al. (1978)	118 / 58 / 60	60	Japan	CL(P)	Lateral
Nakasima & Ichinose et al. (1984)	502 / 251 / 251	220	Japan	CL, CLP, CP	Lateral, PA
Nakasima & Ichinose et al. (1984)	104 / 52 / 52	106	Japan	CL(P)	Lateral, PA
Prochazkova & Tolarova (1986)	40 / 20 / 20	75	Czechoslovakia	CP	Lateral
Word et al. (1989)	82 / - / -	-	USA	CL(P)	Lateral
Sato et al. (1989)	100 / 50 / 50	30	Japan	CL(P), CP	Lateral, PA
Raghavan et al. (1994)	76 / 38 / 38	48	India	CL(P)	Lateral, PA

Prochazkova & Vinsova (1995)	110 / 52 / 52	75	Czechoslovakia	CP	Lateral
Mossey et al. (1997)	83 / 40 / 43	0	UK	CL(P), CP	Lateral
Mossey et al. (1998a)	83 / 40 / 43	100	UK	CL(P), CP	Lateral
Mossey et al. (1998b)	83 / 40 / 43	99	UK	CL(P), CP	Lateral
Suzuki et al. (1999)	65 / 25 / 40	826 (+165)	Japan	CL(P), CP	Lateral, PA
Al Emran et al. (1999)	80 / 40 / 40	67	Saudi Arabia	CL±, CP	PA
Present Study	60 / 30 / 30	30	India	CLP	Lateral

DISCUSSION

Cleft lip and/or palate (CL/P) belong to the most frequent congenital malformations of the face as they can be the result of genetic predisposition affected by some environmental factors. The current study intended to examine the craniofacial morphological characteristics of parents who have children with orofacial cleft and compare this to the parents who do not have children with clefts to determine phenotypic variations which can explain genetic predisposition. Although multifactorial threshold (MFT) model has been traditionally applied to describe the etiology of CL/P, recent views point to the superiority of etiological heterogeneity concept in describing genetic contributions, which are elaborate and inconsistent across families [11].

Studies have established that in affected individuals, the unaffected family members commonly share features in craniofacial constitution. Such characteristics as lengthening of the mandible, changes in cranial base angles, and an increment in anterior facial height are not definitive factors that alone propensity the appearance of a cleft yet may fall as indicators of a genetic vulnerability in combination with environmental factors[12]. Research on the craniofacial morphology of the parents of cleft children has demonstrated that these phenotypic manifestations could be secondary vulnerability markers [13,14]. The results are in tandem with the theory that craniofacial morphologies in parents (phenotype) may play role in the hereditary transfer of cleft related malformations to children (genotype).

In the present research, the fathers having cleft children who did not form a parental pair pairing had various remarkable varying aspects with the fathers in the control group. They were characterized by a considerably smaller symphysis area (p = 0.025), a sharper SNB angle (p = 0.045), significantly smaller mandibular (p = 0.000) and maxillary area (p = 0.001), longer sella-nasion (SN) and a sharper cranial base angle (p = 0.002). These same results agree with those of other studies that observed smaller mandibular size and abnormal cranial base angles as discriminators in the cleft prone groups [15,16].

The mothers of cleft children in comparison with control mothers had considerable differences that were highly-differential such as increased mandibular length (p = 0.000), enlarged anterior facial height (p = 0.000), sharper cranial base angle (p = 0.002) and longer SN length (p = 0.000). These characteristics were pointed in previous works as the distinguishing features of maternal contribution to cleft susceptibility [17]. Especially, the a-angle of the cranial base and the length of the mandible were linked with derailment of timing and position in palatal shelf fusion, which can be stronger in female embryos since the establishment is delayed [18].

In comparison of fathers of the cleft parental pair with control fathers, the results found out a markedly low symphysis area (p = 0.047), extremely low mandibular area (p = 0.000), short palatal length (p = 0.003), low maxillary area (p = 0.027), long SN length (p = 0.018) and steep cranial base angle (p = 0.016). The sequential decreases in the measurement of mandibular and maxillary can disrupt the spatial orientation needed to achieve normal development of the palate [19].

Compared with control mothers, they had a bigger total mandibular length (p = 0.000), anterior facial height (p = 0.005), SN length (p = 0.000), a smaller cranial base

angle (p = 0.000), and longer clivus length (S-Ba) (p = 0.000). With these results to back it up, notions brought forward previously indicate female craniofacial anatomy, and particularly in mothers, might be considered central to cleft pathogenesis [20]. The maternal genome may have a stronger effect on the craniofacial pattern thus raising the chances of defects in the shelf elevation.

Once the gender-specific craniofacial differences in cleft-affected cleft parents have been determined, a discriminant analysis was used to determine parameters that made a significant difference between the cleft and controls. The five significant discriminating characteristics among the fathers were the total mandibular area, the cranial base angle, length of palate, length of the SN and the area of symphysis. Conversely, the lowest number of parameters was only two, which could be used to differentiate groups among mothers, with total mandibular length being the most notable one. These findings reflect on a sexually dimorphic cleft propensity manifestation in craniofacial features [21].

The statistical method of Mahalanobis distance was used to quantify deviation of individual subjects around multivariate mean of their particular groups. The results of the analysis showed that the difference of facial parameter of cleft and non-cleft parents was of great significance. These results underline the necessity to consider cephalometric characteristics of parents according to gender, because with data combination it is possible to mask essential differences[22]. Moreover, although not included in this analysis, it would be possible to cluster the individuals based on their common characteristics and therefore such an approach might reveal new phenotypic groups in the cleft-susceptible population.

Other authors have similarly repeated the importance of these discoveries. According to Figalova, Blanco and Mossey et al., mothers of children with CL/P revealed a significant difference in the mandibular length[17]. Although the direct correlation with this feature and the development of the cleft is not yet clear, it is estimated the higher occurrence of cleft palate in females indicates the maternal craniofacial influence possibility. It can be supposed that the late elevation of palatal shelves in the female embryo renders them more susceptible to derailments due to structural features inherited.

This hypothesis can also be proved with biological evidence. Transforming Growth Factor Alpha (TGFA) and signaling molecule like retinoic acid are some of the genes that have been highly associated with craniofacial development and cleft pathogenesis[21].These morphogens that govern facial growth and merging of tissues might influence any phenotypic patterns of the craniofacial inherited exclusively by one parent or both of the parents.

Although it has provided these insights, there are certain limitations involved in the study. The rather small size of the sample narrows the opportunity to generalize applied findings, and only a few numbers of cephalometric parameters were examined. Broader collection of craniofacial measures and 3D morphometrics imaging would probably be more informative. Also the genetic contribution due to other members of the extended family in addition to the parents was not taken into account which could also be a factor in the transmission of the cleft.

An extended examination of microform cleft traits ought to be done in extended family members to map out the full picture of genetic/phenotypic expression. Determining craniofacial features as potential predisposing factors, which only lead but do not directly result in clefts, like enamel or ear shape anomalies, would prove paramount of investigating the interaction of the genes with the environment. Cases of clefts are also expected to be minimized by preventative measures such as dietary change among the at-risk mothers.

CONCLUSION

To conclude, the present study gives strength to the idea that clear craniofacial characteristics of parents of cleft children can be considered as the phenotypic markers of genetic predisposition. Such identifications may come in handy whenever dealing with a potential parent, especially where incidence of orofacial clefting is high in a community. These results can ultimately become a component of a clinical model including lateral cephalometric analysis, which can provide medical workers with an opportunity to determine a cleft risk and serve their future contribution to prevention.

DECLARATIONS

No funding was received from any financially supporting body, and there was no associated grant number. No funder was involved in manuscript writing, editing approval, or decision to publish.

Consent for publication

Informed consent was obtained from every participant for documentation and examination.

Competing interests

The authors declare no competing interests.

Ethical approval

Ethical approval was granted by the Institutional Human Ethical Committee

Informed patient consent

All patients' clinical records were obtained with informed consent.

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ORIGINAL ARTICALE

OSTEOPROTEGERIN IN PERIODONTAL DISEASE A CROSS-SECTIONAL STUDY

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ABSTRACT

Background: Under both normal and pathological conditions, the RANKL-OPG (Receptor Activator of NF-κB Ligand-Osteoprotegerin) system is an essential regulator of osteoclastogenesis and bone resorption. In order to evaluate OPG's potential as a diagnostic biomarker for periodontal disease, this study aimed to assess osteoprotegerin levels in the gingival crevicular fluid of patients with chronic periodontitis, including those with diabetes mellitus, smokers, and systemically healthy individuals.

Materials and Methods: The study included 60 subjects of age group above 18 years and distributed into 3 groups. GCF was collected and samples were analyzed for OPG concentration using ELISA assay.

Results: No significant difference in PI and GI between 3 groups. PPD shows significant difference between smokers and diabetes with periodontitis. Concentration of OPG is lower in diabetes milletus and smokers when compared to healthy periodontitis

Conclusions: OPG is the marker for bone resorption and can show negative correlation with the clinical parameters like PPD, CAL, thus its increase in levels would denote lesser bone resorption.

Keywords: Osteoprotegerin (OPG), Periodontal disease, Diabetes mellitus, Gingival crevicular fluid, ELISA, RANKL, Probing pocket depth, Clinical attachment loss, Alveolar bone resorption, Cytokines, Bone remodeling, Risk factors.

INTRODUCTION

Periodontitis being a longstanding inflammatory condition affecting the supporting tissues of teeth. Although plaque is the primary cause, periodontal disease is worsened by the factors like genetics and environment. Periodontitis is marked by periodontal pocket formation and loss of alveolar bone.

Under normal health conditions, host immune system controls the presence of bacteria through various mechanisms. However, when the equilibrium in host response and subgingival biofilm is disrupted, it triggers

innate, inflammatory, and adaptive responses. These processes lead to the loss of alveolar bone and periodontal tissue support.

The activation of B and T lymphocytes, significantly affects bone loss in periodontitis. These cells are the main sources RANKL activator during periodontal inflammation.

Th1 cells increase interferon-γ levels, which is a crucial factor in the initiation and progression of periodontitis. Th1 cells release cytokines, TNF-α and IL-1β. These cytokines enhance production of chemokines, which facilitates neutrophil activation and increases MMP release.

Both the soluble and membrane-bound forms of RANKL are produced by activated T cells and B cells. RANKL, a cytokine belonging to the TNF family, stimulates osteoclast differentiation and activation, resulting in resorption of bone.

Important molecules in controlling bone metabolism include RANKL, RANK and OPG.

RANKL by interacting with its receptor RANK, promotes the recruitment of osteoclasts and its precursors onto the surface of the bone.

Osteoprotegerin (OPG) is capable of blocking RANKL's biological functions through competitive inhibition.

High alveolar bone resorption in periodontitis is indicated by an elevated RANKL/OPG ratio.

Studies by Belibasakis et.al have shown that increase RANKL/OPG ratio have been seen in the patients who are smokers.1 Thus, smoking being a risk factor for causing periodontal disease. Apart from various other mechanisms, the effect of vasoconstriction, changes in inflammatory reaction leading to periodontal destruction. This increases the levels of RANKL and decreases the levels of OPG.

Diabetes being a chronic metabolic disorder with higher blood glucose levels, or hyperglycemia, as well as abnormalities in protein, lipid, and carbohydrate metabolism brought on by defect in insulin synthesis or its action or both.

A 2-way relationship exists between diabetes mellitus and chronic periodontitis. Diabetic individuals are 2-3 times more likely to develop periodontitis compared to non-diabetic individuals, and glycemic control is a crucial factor in assessing this risk. The important risk factors for periodontitis include and smoking and diabetes.

Periodontal tissues get accumulated with AGEs and the interaction with their receptor (RAGE, primarily found on macrophages) trigger local immune and inflammatory responses. This leads to increased levels of proinflammatory mediators like IL-6, IL-1β and TNF-α. A disturbance in the RANKL, OPG axis favors bone resorption.

AIMS OF STUDY

To evaluate and co-relate the amounts of osteoprotegerin (OPG) in the crevicular fluid of atients with periodontitis, smokers and diabetic milletus patients.

METHOD OF STUDY

The present clinico-biochemical research study was designed and performed at Department of Periodontics. The study included 60 subjects of age group above 18 years and distributed into 3 groups (**Group -A chronic periodontitis who are systemically healthy), Group -B individuals with periodontitis and diabetes, Group-C smoker patients with periodontitis)** based on inclusion criteria. (i)Patients who are systemically healthy with periodontitis. (ii)Patients who have type 2 controlled diabetes mellitus with periodontitis. (iii) Smokers with periodontitis;(iv) Patients with periodontitis who have minimum two teeth in each quadrant with probing depth ≥5mm;(v) Patients with clinical attachment loss ≥ 3mm; (vi)Patients with evidence of bone loss seen on radiological assessment.

The following were the exclusion criteria:-(i) Uncooperative patients;(ii) Patients who have received periodontal therapy within the last three months. ;(iii) Systemic diseases other than diabetes mellitus;(iv) Use of antibiotics in past 3 months;(v) patients with alcohol and drug consumption.

All subjects in the study met the study criteria and the study design received approval from the Institutional Ethical Committee, and written consent was signed by all the participants.

Clinical examination

A detailed case history was recorded including the Gingival Index (Loe.H and Silness.J, 1963); the Plaque Index (Silness.J and Loe.H, 1964); the UNC-15 periodontal probe for measuring the Probing Pocket Depth (PPD); and Clinical Attachment Loss (CAL).

GCF Sampling

Collection of GCF samples was done using micro pipettes ranging 1 to 5 µl. Micropipettes contaminated with saliva and blood were thrown away. As soon as possible, the collected GCF was placed in aliquots and kept cold until the assay was scheduled.

Measurement of OPG in GCF samples:

GCF samples were analyzed for OPG concentration using ELISA assay. The GCF samples, proteins were quantified using an OPG ELISA development kit. For every assay, the manufacturer's instructions were followed and the OPG concentration is expressed in picograms per milliliter (pg /ml).

Statistical analysis:

All the data obtained from the clinical examination and OPG measurements using ELISA were tabulated against respective subjects using excel sheets, descriptive

analysis was done using mean and standard deviation for which inferential statistics was applied that includes one-way ANOVA with Tukey HSD analysis. The results thus obtained is shown below in the following tables.

RESULTS

Table 1 shows the mean, standard deviation (SD) and 95% confidence intervals (CI) for clinical parameters across the chronic periodontitis, diabetes mellitus with periodontitis and smokers with periodontitis groups. Plaque index (PI) and Gingival index (GI) scores the highest in the smoker with periodontitis group (mean I: 1.17; SD: 0.22; mean GI: 1.05; SD: 0.47) and were slightly lower in diabetes mellitus with periodontitis (mean PI: 0.98; SD: 0.23; mean GI: 1.15; SD: 0.39) and chronic periodontitis (mean PI: 0.96; SD: 0.22; mean GI: 1.20; SD: 0.38). Probing pocket depth was significantly greater in smokers with periodontitis mean (5.78; SD 0.47) and clinical attachment level (CAL) with higher values in smokers (mean 4.19; SD 0.48) than the diabetes with periodontitis and chronic periodontitis. Table 2 shows the mean, standard deviation (SD) and 95% confidence intervals (CI) for Osteoprotegerin (OPG) concentration in gingival crevicular fluid in the respective groups. OPG concentration is highest in chronic periodontitis group (mean 91.38 pg/mL; SD 4.15), significantly lower in diabetes mellitus with periodontitis (mean 26.37 pg/mL; SD 1.78), and in smokers with periodontitis (mean 28.44 pg/mL; SD 6.72). Table 3 shows the comparison of OPG between the groups were the mean OPG for diabetes mellitus with periodontitis was significantly lower than chronic periodontitis ($p < 0.001$). Furthermore, smokers with periodontitis showed significantly lower OPG than the chronic periodontitis ($p < 0.001$). However, there was no significant difference in OPG between diabetes milletus with periodontitis and smokers with periodontitis ($p = 0.349$).

Table 1. Mean, standard deviation (SD) values for plaque index (PI), gingival index (GI), pocket probing depth (PPD), clinical attachment level (CAL) in 3 groups

Clinical parameters	Chronic periodontitis (n=20)				Diabetes milletus (n=20)				Smokers (n=20)			
	Mean	95% CI mean		SD	Mean	95% CI mean		SD	Mean	95% CI mean		SD
		upper	Lower			upper	lower			upper	lower	
PI	0.960	1.062	0.858	0.219	0.980	1.085	0.875	0.225	1.170	1.354	0.987	1.700
GI	1.204	1.383	1.026	0.381	1.146	1.374	0.918	0.486	1.047	1.217	0.877	1.833
PPD	5.191	5.358	5.025	0.355	5.182	5.398	4.967	0.460	5.779	6.077	5.480	6.860
CAL	3.647	3.871	3.422	0.480	3.544	3.738	3.350	0.414	4.194	4.553	3.835	5.610

Table 2. Mean, standard deviation (SD) for the Osteoprotegerin (OPG) measurements from the GCF samples of respective group.

Variable	Chronic periodontitis (n=20)				Diabetes milletus (n=20)				Smokers (n=20)			
	Mean	95% CI mean		SD	Mean	95% CI mean		SD	Mean	95% CI mean		SD
		Upper	Lower			upper	lower			upper	lower	
OPG (pg/ml).	91.380	93.322	89.438	4.150	26.370	27.202	25.538	1.777	28.435	31.580	25.290	6.72

Table 3. Osteoprotegerin (OPG) values compared in all 3 groups. Here the comparison is done by one-way ANOVA and Tukey HSD analysis was applied.

comparison	Mean Difference	SE	T	p value
Group C - Group A	-62.945	1.478	-42.584	<0.001
Group C - Group B	2.065	1.478	1.397	0.349
Group A - Group B	65.010	1.478	43.982	<.001

DISCUSSION

In this study, we analyzed GCF concentrations in healthy individuals, those with diabetes mellitus, and smokers with periodontitis, following assessment of their clinical parameters. Plaque and Gingival indices showed no significance with mean value being 0.960 in Group A,0.980 in Group B,1.170 in Group C as shown in Table 1. Patino Marin [2] and Mazhari et al. [3] found no significant difference in plaque index (PI) between diabetics and non-diabetics. In contrast, Siudikiene et al.[4] demonstrated that the PI in diabetics was significantly higher than in non-diabetics. Buduneli et al.[5] found no significant difference in the mean plaque index (PI) between smoking & non-smoking individuals when oral hygiene levels were comparable.[2] In contrast, Bergstrom et al. (1991)[6] proposed that tobacco has a direct impact on periodontal health, regardless of plaque infection. Thus, systemic influences on the plaque deposition would have much lesser effect than the effect of plaque control measures that have direct effect on the quality and quantity of the plaque deposition.

Probing pocket depth shows significant difference between smokers with periodontitis and diabetes with periodontitis (p value < 0.05.) mean PPD values 5.779 in smokers and 5.182 in diabetes group. On the other hand, there is no significant difference in PPD between healthy (PPD 5.191) and diabetes mellitus subjects (PPD 5.182) P value > 0.05 (table 1). The inference of these finding would be that smoking might cause more destruction of the periodontium when compared to the systemic effects of increased glycemic control.

Likewise, because the probing pocket depth increases would also influence the clinical attachment levels directly, thus CAL when compared between three groups shows significant difference between smokers with periodontitis and diabetes milletus with periodontitis group with CAL values 4.194 and 3.544 respectively P value < 0.05. Significant difference was not found in CAL among group A and group B, with values 3.647 and 3.44, respectively (p > 0.05) (Table 1).

In our present study mean concentration of OPG is lower in group B (diabetes milletus and periodontitis) at 26.370 pg/µl and group C (smokers and periodontitis) at 28.435pg/µl than group A (healthy periodontitis) which was at 91.380pg/µl as seen in table 2. Xin-Ran Xu et al.,[7] compared gingival crevicular fluid (GCF) and serum inflammatory mediators in patients with periodontitis, type 2 diabetes mellitus (T2DM) and healthy individuals. The research found notable high levels of Interleukin-17 and the RANKL/OPG ratio in the diabetes-associated periodontitis (DC) group compared to both the periodontitis (CP) group and the healthy individuals, elevated levels also found in the CP group compared to the healthy group. Although precise concentrations of RANKL and OPG in GCF varied across different studies, a significant hike in RANKL/OPG ratio in periodontitis cases compared to healthy controls was consistently reported, which aligns with the findings of our study.

Bostanci et al.[8] observed a correlation among RANKL/OPG ratio in GCF and probing depth (PD) in periodontitis patients. Similarly, Tabari et al. found positive correlations between attachment loss (CAL) and plaque (PI) with salivary levels of soluble RANKL (sRANKL), the sRANKL/OPG ratio. Our study results are similar to that reported by Bostanci and Tabari et al.

Our study was conducted to know variation in OPG level which is the chemical mediator, that is released from the resident cells in the connective tissue of the periodontium. Cells like fibroblasts, chondroblasts and osteoblasts have been shown to release this cytokine that acts by acting as a decoy for the RANK receptor present on the cells of the haemopoietic linage that acts as a precursors to the osteoclasts, the cells that has these RANK receptors on their cell membrane when activated by the ligand RANKL can lead to their differentiation to form themselves into bone resorbing cells osteoclasts, the action of RANK can not only lead to differentiation but also their further proliferation and their function by secreting various enzymes which are important in the resorption of the bone. Thus, a protective mechanism exists wherein the OPG will selectively bind on these RANK receptors giving no further space for the attachment of RANKL and its functions.

Hence, we tested the presence of this chemical mediator OPG and their relation to the risk factors that are associated strongly with periodontal destruction i.e.; smoking and diabetes milletus levels hypothesizing that their actions will have an effect on the levels of OPG and GCF was collected using micropipettes. As seen in tables 1,2,3 periodontal destruction is significantly more in Group C when compared to Group B patients but significantly lesser in the Group A subjects. Likewise, when biochemical analysis was done to check the levels of OPG it showed significantly higher in Groups C and B subjects than Group A subjects, thus denoting the effects of risk factors that can cause periodontal destruction corelating the significant destruction of periodontal tissue with the decrease in the levels of OPG.

If we would have chosen a larger sample size or addition of more risk factors as groups or a longitudinal study design, a cohort study design would have got a better robust result and thus we consider the above listed as the limitations of this study.

CONCLUSION

We would like to say that OPG is the marker for bone resorption and can show negative corelation with the clinical parameters like PPD, CAL, thus its increase in

levels would denote lesser bone resorption. Also, the risk factors that cause increased periodontal destruction like smoking and diabetes could be due to the decrease in levels of osteoprotegrin.

DECLARATIONS

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ORIGINAL RESEARCH

THE ROLE OF PROPHYLACTIC ANTIBIOTICS IN PEDIATRIC ORAL SURGERY: A SYSTEMATIC LITERATURE REVIEW

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ABSTRACT

The use of prophylactic antibiotics in pediatric oral surgery has been a subject of considerable debate within the medical community . This systematic literature review aims to evaluate the effectiveness, risks, and current recommendations associated with the use of prophylactic antibiotics in pediatric patients undergoing oral surgical procedures. A comprehensive search of relevant databases was conducted to identify studies assessing the outcomes of prophylactic antibiotic administration in pediatric oral surgery, including infection rates, complications, and overall surgical success. The findings suggest that while prophylactic antibiotics may reduce the risk of postoperative infections in certain high-risk pediatric populations, their routine use in all pediatric oral surgeries is not universally recommended. This review discusses factors influencing the decision to administer antibiotics, including the type of procedure, the patient's health status, and existing guidelines. The results highlight the importance of tailored antibiotic use to balance the benefits of infection prevention with the risks of antibiotic resistance and adverse effects. Further research is needed to establish definitive protocols that can guide clinical practice in pediatric oral surgery.

Keywords: Antibiotics, Infection, Pediatric, Prophylaxis, Surgery

INTRODUCTION

Pediatric oral surgery is a specialized field that involves the diagnosis and surgical management of various conditions affecting the teeth, jaws, and associated structures in children [1]. Oral surgical procedures in pediatric patients range from routine extractions of primary teeth to more complex interventions, such as the removal of impacted teeth or treatment of congenital or acquired abnormalities [2].

Given the delicate nature of pediatric patients' developing immune systems, preventing postoperative infections is a significant concern. This has led to the widespread use of prophylactic antibiotics in pediatric oral surgery, aimed at reducing the risk of infection and promoting faster healing [3].

Prophylactic antibiotics are defined as antibiotics administered prior to a surgical procedure to prevent infections. The primary rationale for their use in oral surgery is to counteract the potential for bacterial contamination during surgical interventions, particularly in procedures where there is significant exposure of the mucosa or bone [4]. The need for prophylactic antibiotic therapy is particularly emphasized in patients with underlying medical conditions, such as those with compromised immune systems or those undergoing extensive surgeries. In these cases, the use of antibiotics is often considered a standard practice to avoid postoperative infections, which could lead to more severe complications [5].

However, the routine use of prophylactic antibiotics in pediatric oral surgery is not without controversy. A growing body of evidence suggests that antibiotics may not always be necessary for every surgical procedure and that their indiscriminate use may contribute to the development of antibiotic resistance, a significant public health concern [6]. Antibiotic resistance occurs when bacteria evolve to resist the effects of drugs that once killed them, leading to infections that are more difficult to treat. Additionally, antibiotics can cause adverse side effects, such as gastrointestinal disturbances or allergic reactions, which may complicate the postoperative recovery process. Therefore, it is crucial to carefully evaluate the need for prophylactic antibiotics in each case [7].

Current guidelines on the use of prophylactic antibiotics in pediatric oral surgery vary between different medical organizations. They are often based on expert consensus rather than high-quality randomized controlled trials. The American Academy of Pediatric Dentistry (AAPD) and the American Dental Association (ADA) have published guidelines outlining the conditions under which antibiotics should be used, with a primary focus on high-risk patients and specific surgical procedures. For example, children undergoing invasive procedures, such as the removal of impacted teeth, or those with certain medical conditions, such as heart disease or diabetes, may benefit from prophylactic antibiotics to reduce the risk of infection [8].

Despite these recommendations, there is a lack of consensus on the broader application of prophylactic antibiotics in pediatric oral surgery. The current literature on this subject presents conflicting findings regarding the efficacy of antibiotics in preventing infection in routine pediatric oral surgeries [9]. Some studies indicate that prophylactic antibiotics are beneficial in preventing postoperative infections. In contrast, others suggest that the incidence of infection is low enough in healthy children undergoing routine procedures that antibiotics may not be necessary. Moreover, the risk of antibiotic resistance associated with their overuse adds another layer of complexity to the decision-making process [10].

This systematic literature review aims to critically examine the role of prophylactic antibiotics in pediatric oral surgery, assessing the effectiveness, risks, and current guidelines. By synthesizing evidence from various studies, this review seeks to provide insights into when and why antibiotics should be used in pediatric oral surgery, with a focus on optimizing patient care while minimizing potential harm.

METHODOLOGY

This systematic literature review aims to evaluate the role of prophylactic antibiotics in pediatric oral surgery by synthesizing findings from relevant studies. The methodology followed a structured approach, including clear inclusion and exclusion criteria, a comprehensive search strategy, data extraction, and quality assessment. Below is an outline of the methodology used to gather and analyze the data for this review.

Search Strategy:

A comprehensive search was conducted across multiple electronic databases, including PubMed, Google Scholar, Cochrane Library, and Scopus, to identify studies on the use of prophylactic antibiotics in pediatric oral surgery. The search terms included combinations of "prophylactic antibiotics," "pediatric oral surgery," "infection prevention," "postoperative infection," "children," and "oral surgery." The search was limited to articles published in English, and no date restrictions were applied to ensure a broad capture of relevant studies.

Inclusion Criteria:

Studies included in the review met the following criteria:

- **Population**: Pediatric patients (ages 0-18) undergoing oral surgical procedures.
- **Intervention**: Administration of prophylactic antibiotics prior to or during the oral surgery.
- **Outcome Measures**: Studies reporting postoperative infection rates, complications, or antibiotic-related adverse events.
- **Study Design**: Randomized controlled trials (RCTs), cohort studies, case-control studies, and systematic reviews were included.
-

- **Language**: Articles written in English.

Exclusion Criteria:
Studies were excluded if:

- They involved adult patients or did not specify pediatric populations.
- They focused on dental procedures not involving surgery (e.g., routine cleanings or non-invasive procedures).
- They lacked sufficient data on infection outcomes or complications.
- They were not peer-reviewed or were unpublished conference abstracts.

Data Extraction:
For each study included in the review, the following data were extracted:

- **Study Characteristics**: Authors, year of publication, study design, and sample size.
- **Patient Characteristics**: Age range, medical history, and type of oral surgical procedure.
- **Intervention**: Type of prophylactic antibiotic administered, dosage, and timing of administration.
- **Outcomes**: Postoperative infection rates, complications, adverse effects, and the conclusions drawn by the study authors.

Quality Assessment:
Each study was assessed for methodological quality using appropriate criteria based on the study design:

- **Randomized Controlled Trials** (RCTs) were assessed using the Cochrane Risk of Bias Tool, evaluating factors such as randomization, blinding, and attrition bias.
- **Cohort and Case-Control Studies** were assessed using the Newcastle-Ottawa Scale, which evaluates selection bias, comparability, and outcome assessment.
- **Systematic Reviews** were assessed using the AMSTAR (A Measurement Tool to Assess Systematic Reviews) checklist, which evaluates the comprehensiveness and transparency of the review process.

Final Selection of Studies:
Six studies were ultimately selected for inclusion in this review based on the above criteria. These studies were chosen for their relevance to the topic, the quality of the methodology, and the comprehensiveness of the data they provided regarding the role of prophylactic antibiotics in pediatric oral surgery.

The Prisma Flowchart of the study is shown in Figure 1.

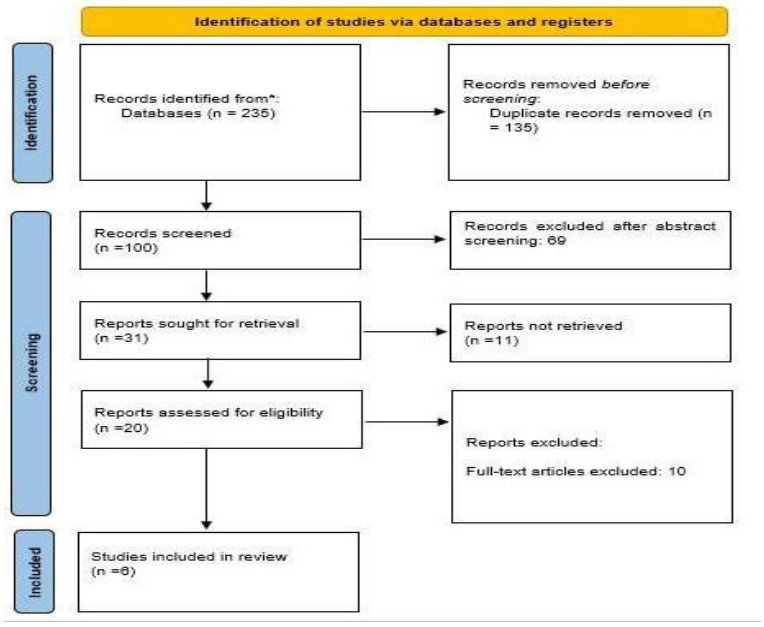


Figure 1. The Prisma Flowchart of the study

Review

The role of prophylactic antibiotics in pediatric oral surgery reveals an ongoing shift in clinical practice and research towards more conservative and evidence-based approaches. Traditionally, antibiotics have been widely used in dental and oral surgical procedures to prevent infection. Still, recent studies suggest that their routine use, particularly in pediatric patients, may not always be necessary. Research from various studies underscores the importance of individualized patient care, where antibiotics are prescribed based on specific risk factors, rather than as a standard practice [8-12].

Table 1. Literature Review of the study

Authors	Reference Number	Year	Journal Name	Findings
Rademacher, W. M. H., Walenkamp, G. H. I. M., Moojen, D. J. F., Hendriks, J. G. E., Goedendorp, T. A., & Rozema, F. R. (2017)	9	2017	Acta Orthopaedica	This systematic review emphasizes that antibiotic prophylaxis is not recommended for preventing periprosthetic joint infections prior to dental procedures. Updated guidelines from the Dutch Orthopaedic and Dental Societies recommend against routine use, instead advocating for reliance on other infection control measures.
Autore G, Bernardi L, Ghidini F, La Scola C, Berardi A, Biasucci G, Marchetti F, Pasini A, Capra ME, Castellini C, et al. (2023)	10	2023	Antibiotics	This guideline discusses the prevention of urinary tract infections in pediatric patients through the administration of antibiotics. The study emphasizes the importance of structured protocols and evidence-based recommendations for antibiotic prophylaxis, with a focus on reducing infection risk in children with urinary tract conditions.

Cuevas-Gonzalez, María Verónica PhD, Cuevas-Gonzalez, Juan Carlos PhD, Espinosa-Cristóbal, León Francisco PhD, et al. (2023)	**11**	**2023**	**Medicine**	This study systematically reviews the use of antibiotics as prophylactic therapy in oral surgery, highlighting a lack of sufficient evidence for their routine use. The authors advocate for a more cautious approach, calling for updated strategies in clinical practice and suggesting alternative infection control methods in oral surgery.
Vidović Juras D, Škrinjar I, Križnik T, Andabak Rogulj A, Lončar Brzak B, Gabrić D, Granić M, Peroš K, Šutej I, Ivanišević A. (2024)	12	2024	Dentistry Journal	This article examines the diverse practices in dental antibiotic prophylaxis and emphasizes the importance of evidence-based recommendations for antibiotic use before dental procedures. The study evaluates different approaches in practice and stresses the need for further research to establish uniform protocols.
Esposito S, Sgarzani R, Bianchini S, Monaco S, Nicoletti L, Rigotti E, Di Pietro M, Opri R, Caminiti C, Ciccia M, et al. (2022)	13	2022	Antibiotics	This study uses the RAND/UCLA Appropriateness Method to assess the necessity of surgical antimicrobial prophylaxis for pediatric patients undergoing plastic surgery. It provides a consensus among experts, emphasizing the guidelines for when antibiotics should be administered to prevent infections during post-surgical recovery.

Cuevas-Gonzalez, María Verónica PhD, Cuevas-Gonzalez, Juan Carlos PhD, Espinosa-Cristóbal, León Francisco PhD, et al. (2023)	**11**	**2023**	**Medicine**	**This study systematically reviews the use of antibiotics as prophylactic therapy in oral surgery, highlighting a lack of sufficient evidence for their routine use. The authors advocate for a more cautious approach, calling for updated strategies in clinical practice and suggesting alternative infection control methods in oral surgery.**
Vidović Juras D, Škrinjar I, Križnik T, Andabak Rogulj A, Lončar Brzak B, Gabrić D, Granić M, Peroš K, Šutej I, Ivanišević A. (2024)	12	2024	Dentistry Journal	This article examines the diverse practices in dental antibiotic prophylaxis and emphasizes the importance of evidence-based recommendations for antibiotic use before dental procedures. The study evaluates different approaches in practice and stresses the need for further research to establish uniform protocols.
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Esposito S, Sgarzani R, Bianchini S, Monaco S, Nicoletti L, Rigotti E, Di Pietro M, Opri R, Caminiti C, Ciccia M, et al. (2022)	**13**	**2022**	**Antibiotics**	This study uses the RAND/UCLA Appropriateness Method to assess the necessity of surgical antimicrobial prophylaxis for pediatric patients undergoing plastic surgery. It provides a consensus among experts, emphasizing the guidelines for when antibiotics should be administered to prevent infections during post-surgical recovery.
Mooney, James F. et al. (2023)	14	2023	Journal of the Pediatric Orthopaedic Society of North America	This survey examines the common practices of post-surgical dental precautions and prophylaxis in pediatric patients undergoing oral surgery. It highlights a variety of practices within the pediatric orthopaedic community. It emphasizes the importance of proper dental care following surgery to prevent complications, with a special focus on the use of antibiotics.

Table 1 provides a summary of six studies related to antibiotic prophylaxis in pediatric oral surgery and infection prevention. It includes key details such as the authors, year of publication, journal name, and the central findings of each study. The studies, spanning from 2017 to 2024, emphasize the growing recognition that routine use of antibiotics for prophylaxis in pediatric oral surgery may not always be necessary, with several studies calling for more evidence-based approaches to antibiotic administration. Many studies emphasize the importance of updated guidelines and tailored clinical practices to ensure antibiotics are used appropriately, thereby reducing the risk of antibiotic resistance and improving patient outcomes. By highlighting the varying perspectives and recommendations, the table offers a comprehensive overview of current research on this critical aspect of pediatric surgical care. A key finding in the literature is the lack of robust evidence supporting the routine use of antibiotics for prophylaxis in many pediatric oral surgeries. This has led to updated guidelines that recommend a more judicious approach, aimed at reducing the risk of antibiotic resistance and minimizing unnecessary interventions. Studies have highlighted that while antibiotics are essential in certain high-risk scenarios, such as for patients with compromised immune systems or those undergoing more complex surgeries, for many routine procedures, the risks associated with antibiotic use may outweigh the benefits [11-14].|

Furthermore, the growing concern over antibiotic resistance in healthcare is influencing the way prophylactic antibiotics are prescribed, not just in pediatric oral surgery but across various medical and surgical disciplines. The call for updated clinical guidelines that prioritize the appropriate use of interventions based on a patient's health status, the

type of surgery, and evidence-based practices reflects a broader shift in medical research. As such, the role of prophylactic antibiotics is being reconsidered in light of new data, with an emphasis on patient safety, better infection control strategies, and more effective antibiotic stewardship. This evolving perspective is shaping the future of pediatric oral surgery, moving away from routine antibiotic use and towards more targeted, evidence-driven approaches**.**

Taken together, these studies underline the importance of individualized care in pediatric oral surgery. Prophylactic antibiotics appear to be beneficial in high-risk groups, such as those with underlying medical conditions or undergoing complex surgeries. However, for routine procedures in healthy children, the evidence supporting their widespread use is weak, and the risks of antibiotic resistance and side effects must be carefully considered. More research is needed to develop comprehensive guidelines that strike a balance between the need for infection prevention and the potential harms of overuse.

DISCUSSION

The use of prophylactic antibiotics in pediatric oral surgery is a longstanding practice, aimed primarily at preventing postoperative infections. In pediatric patients, the decision to use antibiotics prior to oral surgical procedures, such as tooth extractions, maxillofacial surgeries, and other invasive treatments, remains a topic of ongoing research and debate [4,5]. While antibiotics have undoubtedly proven effective in reducing infection rates in certain high-risk populations, the growing concern over antibiotic resistance and adverse side effects has spurred calls for more cautious and evidence-based approaches [6].

Historically, prophylactic antibiotics have been administered routinely to all pediatric patients undergoing oral surgery. This was done with the assumption that it would reduce the risk of postoperative infections, which could lead to complications like delayed healing, prolonged recovery times, or even systemic infections. However, as the understanding of infection control and antibiotic resistance has evolved, so too has the approach to prophylaxis. Today, the use of prophylactic antibiotics in pediatric oral surgery is increasingly being scrutinized, with calls for more selective use based on individual patient characteristics and the nature of the surgery [7,8].

One of the primary considerations in determining the necessity of prophylactic antibiotics is the patient's risk profile. Children with pre-existing medical conditions, such as congenital heart defects, diabetes, or compromised immune systems, are more susceptible to postoperative infections and may benefit from antibiotic prophylaxis.

The American Heart Association, for example, has guidelines recommending prophylactic antibiotics for children with specific cardiovascular conditions undergoing dental procedures. In these cases, the use of antibiotics is viewed as a preventive measure against more severe infections, such as infective endocarditis, which could have serious consequences [5-7].

However, for healthy children undergoing routine oral surgeries—such as simple tooth extractions or minor soft tissue procedures—the benefits of prophylactic antibiotics are less clear. Studies have shown that the risk of infection following these procedures is relatively low, even in the absence of antibiotics. In fact, the overuse of antibiotics in these situations may expose children to unnecessary risks, including allergic reactions, gastrointestinal disturbances, and the development of antibiotic resistance. Antibiotic resistance is a growing concern in public health, as the misuse and overuse of antibiotics contribute to the emergence of drug-resistant bacteria, making infections more difficult to treat in the future [4].

The evolving understanding of infection prevention and control also complicates the debate over the routine use of prophylactic antibiotics. Advances in surgical techniques, improved hygiene practices, and better postoperative care have all contributed to a decline in infection rates in pediatric oral surgeries. Furthermore, a more targeted approach, where antibiotics are prescribed only for high-risk patients or complex procedures, may be just as effective without the need for routine administration in every case. The evolving guidelines and expert opinions on the use of prophylactic antibiotics highlight the need for a more individualized approach to pediatric oral surgery. It is increasingly recognized that a "one-size-fits-all" approach may not be appropriate, and the decision to administer prophylactic antibiotics should be based on the patient's health status, the complexity of the procedure, and the presence of any risk factors that might predispose the child to infection.

CONCLUSION

In conclusion, while prophylactic antibiotics continue to play an important role in preventing infections in certain pediatric oral surgical procedures, their routine use in all cases is no longer considered standard practice. The trend in recent years has been toward a more selective, evidence-based approach, ensuring that antibiotics are used judiciously to maximize their benefits while minimizing potential harms. Further research is needed to develop clear and comprehensive guidelines for administering prophylactic antibiotics in pediatric oral surgery, taking into account both the risk of infection and the potential risks associated with

antibiotic use. This will help ensure that children receive the best care possible while preserving the effectiveness of antibiotics for future generations.

DECLARATIONS

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Consent for publication

Informed consent was obtained from every participant for documentation and examination.

Competing interests

The authors declare no competing interests.

Ethical approval

Ethical approval was granted by the Institutional Human Ethical Committee

Informed patient consent

All patients' clinical records were obtained with informed consent.

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ORIGINAL ARTICALE

COMPARATIVE ANALYSIS OF INDIVIDUAL REPRESENTATIVES OF THE MICROBIOTA OF THE PERIIMPLANT ZONE IN NORMAL AND INFLAMMATORY CONDITIONS

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ABSTRACT

Background: The incidence of periimplantitis has increased significantly in recent years, which has led to an increase in the number of implant rejection cases and a deterioration in patients' lives.

The purpose of the study. To conduct a comparative analysis of individual representatives of the microbiota of the peri-implant sulcus in normal and inflammatory conditions.

Materials and methods: Samples of the contents of the peri-implant sulcus from 20 patients with healthy peri-implant tissues and 20 patients with peri-implantation. The quantitative and qualitative composition of the microbiota in the samples was determined by the classical bacteriological method.

Results: It was found that *Streptococcus spp., Staphylococcus spp., Candida spp. and Escherichia coli* predominate in the samples obtained from patients of the main group. Moreover, the number of *Candida spp*. In the main group, the number of *Candida spp* is 20.8 times higher. in the comparison group. In addition, representatives of *Sarcina spp*., which had not previously been associated with the development of peri-implantitis, were found.

Conclusion: The microbiota plays a crucial role in the development of peri-implantitis. Further research aimed at understanding the role of various microorganisms and their mechanisms of action will make it possible to develop effective strategies for the prevention and treatment of this complex disease. Maintaining good oral hygiene and regular routine checkups remain key factors in preventing the development of peri-implantitis.

***Keywords*:** dental implants, peri-implantitis, microbiota, microbial biofilms.

1.INTRODUCTION

The use of dental implants is a highly effective method of restoring the integrity of the dentition in patients with missing teeth. Despite the high degree of success, implantological treatment is associated with the risk of complications, in particular, peri-implantitis. Numerous experimental and clinical studies have shown that peri-implantitis is associated with infection of peri-implanted tissues by microorganisms of the oral cavity due to unsatisfactory individual oral hygiene. Microbial biofilm forms on all surfaces of the implant structures, provoking inflammation followed by destruction of bone tissue [1-5]. The results of clinical studies have shown that the risk of peri-implantitis in patients with periodontitis, both in the active phase and in remission, is five times higher than in patients with healthy periodontitis [6-9].

Despite the opinion about the identity of the microorganisms that cause periodontitis and peri-implantitis, and the detection of similar pathogenic microorganisms in the periodontal pockets and in the area around the implants, a number of authors deny the possibility of infection of the peri-implant zone from periodontal foci of infection. In this regard, the study of the microbiota in the combined course of these diseases is of considerable scientific interest [10].

The pathogenesis of peri-implantitis is less studied, and its features are primarily related to the structural features of the peri-implant zone[1,4]. Canullo L, Penarrocha-Oltra D et al. suggested that the absence of periodontitis in the implant area causes the mobility of the soft tissues of the peri-implant zone. This, in turn, leads to greater traumatization, increasing the adhesive capabilities of microorganisms and contributing to contamination of the peri-implant sulcus [11]. Peri-implantitis, like periodontitis, is an opportunistic disease; therefore, the bacteria that cause it are part of the normal microbiota of the oral cavity [12]. Bacteria from the oral fluid and dental plaque colonize the surface of the implant, colonizing the peri-implantant groove. Different types of microorganisms form associations (biofilms) for joint survival in the oral cavity. Microbes interact closely in biofilms, which leads to an increase in their pathogenicity, mutual provision of nutrients, exchange of genetic information, and formation of intracolonial signaling links, which leads to further progression of inflammation [4,26]. The microbiome of a healthy peri-implant sulcus is characterized by a low ratio of anaerobic and aerobic species and a small number of periodontal pathogens [13]. However, with certain microecological shifts, bacteria associated with inflammation become dominant and, acting in concert, acquire pathogenic properties [12]. At the same time, the anaerobic microbiota creates an acidic environment around the implant, which poses a physico-chemical threat to the stability of the oxide layer of the implant, disrupting the connection of the implant to the gum and bone and leading to the loss of surrounding and supporting tissues of the implant [2].

Despite the recognized priority of biofilms in the etiology of peri-implant diseases, there is still no consensus on the differences in the composition of the submucosal biofilm around implants in normal and inflammatory conditions. A clear understanding of the microbial profiles of the peri-implant sulcus is necessary to understand the consequences of microecological changes and to develop effective strategies for the prevention, diagnosis and treatment of peri-implantitis.

The aims of the study: To conduct a comparative analysis of individual representatives of the microbiota of the peri-implant sulcus in normal and inflammatory conditions.

MATERIALS AND METHODS

The study was conducted on the basis of two clinics in Tver (Russia): Professor Strelnikov's Clinic and Morozovskaya Dentistry, as well as at the Department of Microbiology and Virology with a course in immunology at the Tver State Medical University of the Ministry of Health of the Russian Federation.

The study included 40 patients, including 26 women and 16 men aged 24 to 60 years, who received dental implants of the Dentium system (South Korea). The study was conducted in compliance with all the principles of the Helsinki Declaration and approved by the local Ethics committee of the Tver State Medical University of the Russian Ministry of Health. Before being included in the study, a "Voluntary informed consent for examination" was signed with each volunteer, and an individual dental patient's medical record was created for each patient to collect information about the presence of oral diseases, as well as concomitant acute and/or chronic diseases of internal organs and systems, and an allergic history.

The patients were divided into two groups: group 1 (main) – patients with peri–implantation (n=20), group 2 (comparison) - patients with implants without signs of inflammation of surrounding tissues (n=20). All patients included in the studies did not have a diagnosis of "chronic periodontitis" established earlier and/or at the time of the examination (k05.3).

The examination of patients was carried out according to a generally accepted scheme, including a survey, clinical and X-ray dental examination, including periodontal probing.

Inclusion criteria: (1) age 18 years and older, (2) the presence of at least 1 dental implant, the functioning of which with a load of suprastructure for at least 6 months.

Design of the selection of dental implants included in the study: One dental implant was evaluated for each patient. In the presence of several healthy implants, an implant with the best hygienic and periodontal parameters was selected. In the presence of several affected implants, an implant with the worst hygienic and periodontal parameters was selected to assess the most severe condition. If one patient had both implants with healthy surrounding tissues and peri-implantation, then this

patient was included in group 1 (the main one), and the affected implant was analyzed.

Exclusion criteria: (1) unsigned voluntary informed consent to participate in the study, (2) pregnancy, (3) diabetes mellitus, (4) HIV infection, (5) taking antibacterial drugs for 3 months prior to sampling, (6) patients receiving intravenous and/or oral bisphosphonate therapy, (7) patients who underwent any type of anti-inflammatory therapy for 3 months prior to sampling, (8) the presence of a history of chronic periodontitis according to medical documentation or identified after examination, (9) the depth of probing in the implant area is more than 6 mm.

Sample collection, microbiological monitoring:

The samples for the study were taken from the periimplant sulcus (Fig. 1).

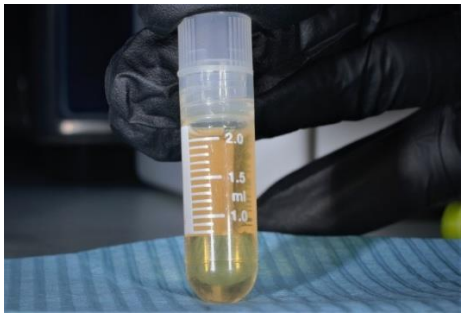


Figure 1. Tubes with Ames transport medium

The material was taken with sterile paper pins, which were placed in the sulcus for 30 seconds, and then into tubes with a special transport medium, Ames medium without coal (HiMedia, India) (Fig. 2).



Figure 2. Sampling of the contents of the peri-implantation sulcus

The test tubes were tightly closed with lids and delivered to the laboratory within 2 hours to isolate pure cultures of microorganisms using the classical bacteriological method. 0.1 ml of the test material was taken for seeding (from the transport medium) and seeded on nutrient media: mannitol salt agar (M118) (HiMedia, India), blood agar (HiMedia, India), Saburo agar with glucose and chloramphenicol (HiMedia, India), streptococcal agar (HiMedia M304) (HiMedia, India), followed by incubation. Smears were prepared from isolated colonies grown on appropriate nutrient media and stained using the Gram method. Morphological and tinctorial properties of microorganisms were studied using the Diamorphocito® software and hardware complex (Russia) (1:1000 magnification using a Biolam binocular microscope). The number of bacteria was determined by counting colony-forming units per 1 ml of the test material (lg CFU/ml). The identification of microorganisms was carried out by biochemical activity using API® test systems (bioMérieux Vitek, Inc.) and API® WEB PC software.

Statistical processing of results:

Statistical characteristics based on samples, hypothesis testing based on statistical criteria was performed using Microsoft Excel [Microsoft Excel 2013, Microsoft Corp., USA]. Statistical differences between the groups were determined using the Student's criterion. Statistical significance was determined at the level of $p < 0.05$ to compare the quantitative composition of the microbiota and at the level of $p < 0.001$ to compare the indicators of dental status.

RESULTS

The results of the assessment of the dental status of patients in the two groups are presented in Table 1. The values of all the studied indicators significantly differed in patients of the first and second groups. The depth of probing in the implant area in patients of group 1 did not exceed 6 mm, in patients of group 2 – 3 mm against the background of general periodontal health. These indicators correlate with the value of the oral hygiene index and correspond to an average "satisfactory" level of oral hygiene for patients in group 1, with individual patients having "unsatisfactory" oral hygiene. In the comparison group, the average hygiene index values corresponded to "good" oral hygiene, and only some patients had a "satisfactory" level of oral hygiene.

In our study, when comparing the results obtained in patients with peri-implantation (the first group, n = 20) and patients in the comparison group (the second group, n = 20), significant differences in the microbial diversity of the contents of the peri-implant sulcus were revealed.

Table 1. The results of the assessment of the dental status of patients in two groups

Indicator	Group 1 (main) n=20			Group 2 (comparisons) n=20			p-value
	M_1	Std_1	Max_1	M_2	Std_2	Max_2	
Depth of periodontal probing (PPD), mm	3,55	1,145	6	2,05	0,686	3	≤0.001
Depth of sensing in the area of implants, mm	4,95	1,234	6	2	0,561	3	≤0.001
The level of radiological bone loss in the area of implants, mm	4,65	1,268	6	0,775	0,734	2	≤0.001
Silness-Loe Oral Hygiene Index, points	1,54	0,949	3	0,52	0,242	1,2	≤0.001
Mühlemann bleeding index, points	1,97	0,989	3,5	0,51	0,35	1,4	≤0.001

The values of all the studied indicators significantly differed in patients of the first and second groups. The depth of probing in the implant area in patients of group 1 did not exceed 6 mm, in patients of group 2 – 3 mm against the background of general periodontal health. These indicators correlate with the value of the oral hygiene index and correspond to an average "satisfactory" level of oral hygiene for patients in group 1, with individual patients having "unsatisfactory" oral hygiene. In the comparison group, the average hygiene index values corresponded to "good" oral hygiene, and only some patients had a "satisfactory" level of oral hygiene. In our study, when comparing the results obtained in patients with peri-implantation (the first group, n = 20) and patients in the comparison group (the second group, n = 20), significant differences in the microbial diversity of the contents of the peri-implant sulcus were revealed (Fig. 3). It was found that the dominant representatives of the microbiota in the studied samples were Peri-implantitis are *Streptococcus spp., Staphylococcus spp., Candida spp. and Escherichia coli* ($p < 0.05$).

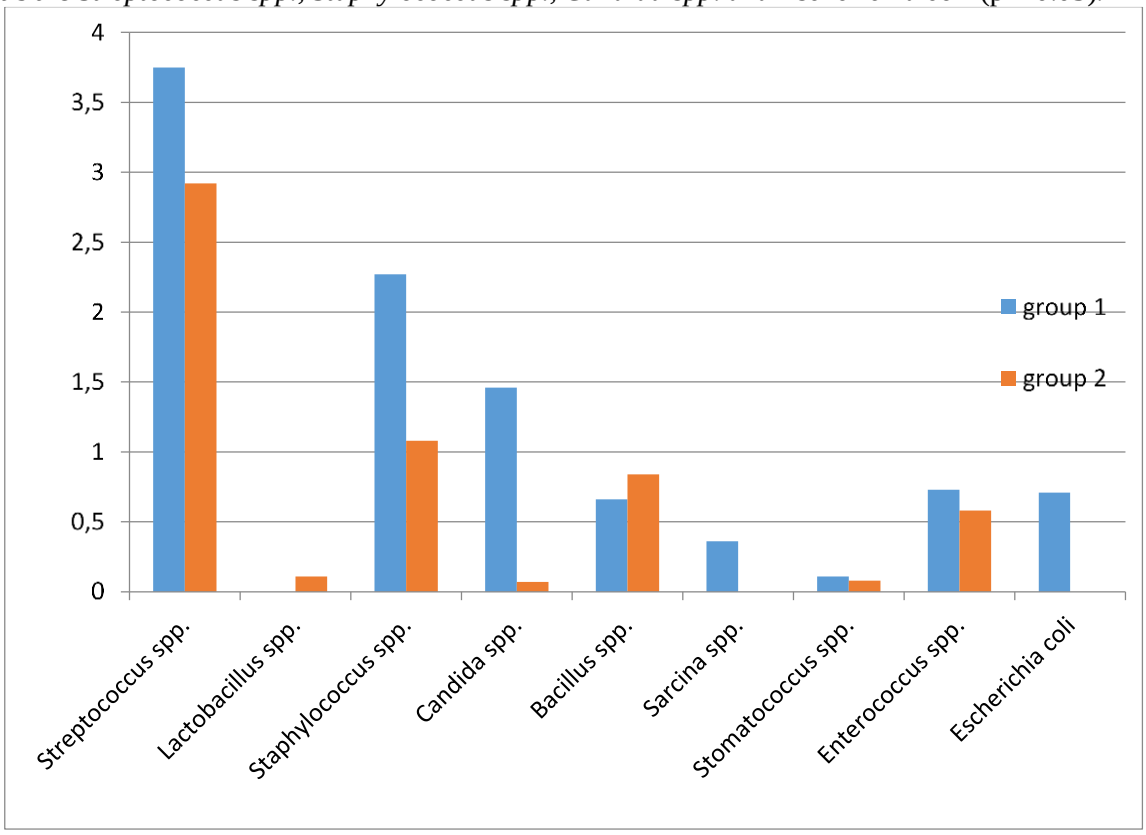


Figure 3. Comparison of the quantitative composition of isolated microorganisms of the main group and the comparison group (lg CFU/ml).

Also, representatives of *Sarcina sp*. were found in the area of the peri-implant sulcus in patients with peri-implantation, with their complete absence in the case of a healthy peri-implant zone. The specific and quantitative composition of the microbiota isolated from the peri-implant sulcus of the patients of the two groups is presented in Table 2.

Table 2. Species and quantitative composition of microorganisms isolated from the periimplant sulcus (lg CFU/ml)

Microorganisms	Group 1 (main) n = 20		Group 2 (comparisons) n =20		p-value
	M_1	Std_1	M_2	Std_2	
Streptococcus spp.	3.75	0.74	2,92	0.85	**0.000441***
Lactobacillus spp.	0	0	0.11	0.50	0.165282
Staphylococcus spp.	2.27	1.14	1.08	0.96	**0,002051***
Candida spp (Candida albicans)	1.46	1.32	0.07	0.31	**0,00009***
Bacillus spp.	0.66	1.24	0.84	1,36	0,340765
Sarcina spp.	0.36	0.89	0	0	**0,042056***
Stomatococcus spp.	0.11	0.49	0.08	0.39	0,164938
Enterococcus spp.	0.73	1.57	0.58	1.26	0,352221
Escherichia coli	0,71	1,42	0	0	**0,018657***

* the differences are statistically significant times greater ($p<0.05$).

DISCUSSION

The results of our research differ from the results obtained in the study by Kensara A et al. [14]. There were no differences in microbial diversity between the healthy peri-implant zone and the area of implants with peri-implant ($p = 0.82$). A controlled cross-sectional clinical trial conducted by Kensara A et al. [15] involved 23 patients: 11 with healthy implants and 12 with affected peri-implantitis. The aim of the study was to characterize the composition of the microbiome in the field of dental implants with peri-implant and healthy implants. It was noted that higher levels of Gram-positive bacteria, especially *Enterococcus spp*, were observed around peri-implanted implants compared to healthy implants. In contrast to this study, our study did not find significant differences in the number of *Enterococcus spp*. between healthy and affected implants.

In addition, Sousa V. et al. [16] proved that in conditions simulating implant health, *Staphylococcus spp*. and *Candida spp*. they are present in smaller quantities. This situation is in sharp contrast to biofilms in peri-implantitis, which are denser, more diverse and contain more pathogenic species, which is consistent with the results of our study, which contains the presence of *Staphylococcus spp*. and *Candida spp*. It was higher in peri-implantitis than in the area of healthy implants.

Candida albicans is a fungal pathogen often involved in various infectious processes of the oral cavity, including peri-implantitis. This opportunistic yeast fungus is able to attach and colonize both mucosal surfaces and inert materials, as well as form biofilms, which is a critical factor in its pathogenicity. Indeed, biofilms protect *Candida albicans* from environmental stresses, including antifungal drugs and the host immune response [4,26,28,29]. Biofilms containing *Candida albicans* provide a protective environment not only for the yeast itself, but also for other representatives of the microbiota, including bacterial pathogens, which complicates treatment and leads to chronic infection [25]. It is important to draw a parallel with the course of periodontitis and endo-periodontal lesions: *Candida albicans* is found in 20% of cases with periodontitis and in 33-55% of cases with reinfected root canals [26]. In the context of peri-implantitis, *Candida albicans* can attach to the implant surface and form biofilms containing a complex community of microorganisms, which contributes to the maintenance and aggravation of the infectious process [27, 28].

However, the research results turned out to be contradictory: in some studies, *Candida* species were detected with the same frequency in both affected and

healthy areas, while in others, with a higher frequency in peri-implantitis areas ($p < 0.05$) [18, 19], which is consistent with the results of our study: the number of *Candida spp*. In the main group, the number of *Candida spp*. is 20.8 times higher. in the comparison group. It is interesting to note that *Candida spp*. It is capable of colonizing even areas of clinically healthy peri-implantant tissues.

The stability and stability of the microbial community in healthy areas around the implant are crucial for maintaining the health of peri-implant tissues [17]. The presence of opportunistic and saprophytic bacterial species associated with conditional oral health, such as *Streptococcus spp.*, probably plays a protective role by preventing colonization and overgrowth of pathogenic species. These bacteria contribute to maintaining a balanced microbial ecosystem that supports healthy tissues and prevents inflammation [20].

Representatives of the genus *Streptococcus spp*. act as "pioneers" in the formation of biofilms, initially attaching to the surface of teeth through the production of extracellular polysaccharides using glycosyltransferases, which, in turn, ensures the attachment of other microorganisms. By producing organic acids such as lactic acid, *Streptococcus spp*. helps regulate the local pH environment [21]. In addition, representatives of the genus Streptococcus produce antimicrobial substances such as hydrogen peroxide, which inhibit pathogenic bacteria, including cariogenic and periodontal microbiota [22, 23]. In addition to these functions, *Streptococcus spp.* It also interacts with the host's immune system to strengthen the integrity of the mucosal barrier and prevent excessive activation of inflammatory reactions [24]. The results obtained in our study show that *Streptococcus spp*. They are present both in the field of healthy implants and in the field of implants with periimplantitis. At the same time, the number of *Streptococcus spp*. in the main group, the number of *Streptococcus spp*. exceeds 1.28 times. in the comparison group, which suggests their role in the transition from health to disease.

A systematic review by Lafaurie GI et al. [29] showed that peri-implant tissues in the area of affected implants are also colonized by gram-negative microorganisms such as *Enterobacteriaceae spp.*, especially Escherichia coli, which are infrequently found in the area of teeth with periodontitis or healthy implants. Similarly, a study by Ardila CM et al. [30] showed that the presence of gram-negative E. coli in the implant area was associated with a deterioration in clinical parameters in patients with peri-implantation ($p <0.001$). In a cross-sectional study, Ramón-Morales CA et al. [31] the presence of *Escherichia* coli has been associated with peri-implantitis. Our results in this study also confirm that in patients with peri-implantitis, the microbial landscape is characterized by the appearance of gram-negative rods, including *Escherichia coli*.

Despite the limitations of this study, it was possible to find some correlations between the presence of *Sarcina* in the peri-implant area and the presence or absence of the disease. Although we cannot conclude that these microorganisms contribute to the development of peri-implantitis, their abundant presence should not be ignored, and their etiological role in peri-implantitis requires further investigation.

The results of our study are consistent with the conclusions of numerous domestic and foreign studies conducted in recent years, which compared the microbiome of healthy implants and implants affected by peri-implantitis. These studies indicate that the biofilm in peri-implantitis is characterized by a greater microbial diversity, a greater microbial load, and a more complex structure compared to healthy areas. [13, 15, 32 - 35].

1. The biofilm of the peri-implant zone during inflammation is characterized by a large microbial diversity and a greater microbial load.
2. The presence of *Candida albicans* in the peri-implant zone during inflammation may be associated with the severity and duration of the course of peri-implantitis.
3. The role of *Sarcina spp*. The development of the infectious process in peri-implantitis requires additional study.
4. The depth of tissue probing in the implant area and the severity of peri-implantitis correlate with the level of oral hygiene.
5. In order to develop effective and scientifically based protocols for the prevention and treatment of tissue pathology surrounding implants, a detailed understanding of the etiology, pathogenesis and diagnosis of these diseases is necessary.

CONCLUSION

The microbiota plays a central role in the pathogenesis of peri-implantitis, and its composition differs significantly from that of the microbiota in the healthy tissues surrounding the implants. Disruption of the balance of microorganisms, biofilm formation, activation of the immune response and metabolic processes caused by bacteria lead to inflammation and tissue destruction in the implant area. A comprehensive analysis of microbiological data is an important tool for understanding the etiology and pathogenesis of peri-implantitis. This will make it possible to develop more effective strategies for the treatment and prevention of peri-implantitis and, as a result, ensure the durability of dental implants.

Limitations of the study. This study is limited to a small sample size and focuses exclusively on the microbiome of healthy and peri-implanted sites. In addition, the main group included patients with a depth of probing in the implant area not exceeding 6 mm. Future research should eliminate these limitations by increasing the sample size and conducting a comparative analysis of microbiome profiles from peri-implant sites (both healthy and

diseased), depending on the method of fixation of orthopedic structures on dental implants.

DECLARATIONS

Ethical approval and consent to participate

Not Applicable

Competing interest

The authors declare that there are no competing interests.

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ORIGINAL RESEARCH

SURFACE AND ELEMENTAL ANALYSIS OF TITANIUM MINISCREW IMPLANTS (FAVANCHOR AND ORLUS) AFTER CLINICAL USE IN ORTHODONTICS: A SCANNING ELECTRON MICROSCOPY AND ENERGY DISPERSIVE X-RAY MICROANALYSIS STUDY

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ABSTRACT

Background: Titanium miniscrew implants (MSIs) are widely used in orthodontics as temporary anchorage devices. However, these implants are susceptible to mechanical and chemical alterations during their clinical use. This study investigates the surface and elemental changes in two brands of titanium miniscrew implants, Favanchor and Orlus, after clinical exposure, utilizing Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) microanalysis.

Methods: Twenty titanium miniscrew implants (10 Favanchor and 10 Orlus) were retrieved after being used in patients for at least six months. Control samples of each implant type were analyzed in their as-received state. SEM was employed to assess the surface morphology, while EDX provided elemental composition data. Flexural strength was also measured using a Universal Testing Machine.

Results: SEM analysis revealed significant surface alterations in the retrieved implants, including dullness, blunting of threads, corrosion, and fractures. The retrieved Favanchor implants showed more severe damage, particularly in the body and tip regions. Elemental analysis indicated the presence of additional elements such as calcium, iron, cerium, and oxygen, which were not present in the as-received implants. The comparison of flexural strength revealed a significant reduction in strength for both implant brands, with Orlus showing less degradation than Favanchor. In particular, the flexural strength for the retrieved Favanchor implants was lower compared to the Orlus implants, indicating more significant mechanical degradation.

Conclusion: Both implant brands exhibited substantial surface degradation and elemental composition changes after clinical use, with Favanchor showing more severe damage. These alterations highlight the need for careful consideration when reusing miniscrew implants in clinical practice, particularly in terms of their structural integrity and elemental composition

Keywords: Corrosion, Energy Dispersive X-ray, Flexural Strength, Scanning Electron Microscopy, Titanium Miniscrews

INTRODUCTION

With the advent of titanium miniscrews transforming the orthodontic landscape in the last two decades, enabled clinicians for the establishment of stable intraoral anchorage to accomplish enhanced therapeutic results [1]. These devices are small, simple for the insertion and removal, thus allowing for immediate loading reducing overall treatment time thus making the treatment economical [2,3]. With these devices, moderate to severe skeletal abnormalities of the patient are able to be overcome with tooth movements previously thought unattainable. Results with mini-screw implant supported anchorage are seen to be more predictable than other types of compliance dependent anchorage. The pioneering of skeletal anchorage use as in the form of Vitallium screw placement in the mandibular rami of dog mandibles can be credited to Gainsforth and Higley [4]. Similarly, in 1983 osteosynthesis surgical bone screws were used for the intrusion of maxillary incisors by Creekmore and Eklund [5]. Specific orthodontic application mini-implants with 1.2mm diameter were introduced by Kanomi in 1997 [6]. MSIs contain grade V titanium alloy - Ti-6Al-4V comprising of 6% aluminium, 4% vanadium, 0.25% (maximum) iron, 0.2% (maximum) oxygen, and Ti which gives it enhanced strength thus making it more durable for it's use in implant placement. Grade 5 machined titanium leads to enhanced cellular adhesion and proliferation with a strong cytocompatibility; yet lacks osseointegration leading to easier removals as necessary for temporary anchorage devices [8,9].

Mechanical failure in the oral environment leads to MSIs being fractured occasionally with causation of gingival injuries as documented which have to be either removed or replaced [10]. The effectiveness is influenced by a number of parameters such as implant macro and microstructure qualities, implantation site characteristics and patient selection [11-13]. Also due to the formation of stable passive titanium oxide layer, they have been observed to undergo corrosion during clinical use [14].

Energy Dispersive Spectroscopy (EDS) and Scanning Electron Microscopy (SEM) are two complementary techniques used to characterize material surfaces. With their combination, analysis of elemental composition and visualization of surface features at high magnification is possible. EDS analyzes the elemental composition of a material by detecting X-rays emitted when the material is irradiated with an electron beam thus providing compositional information on a micro- or nanoscale. SEM uses an electron beam to produce a high-resolution surface image by interaction of electron beam with specimen atoms leading to scattering, leading to the release of electrons and X-rays which are then detected and used to create a surface image [15]. These techniques have diverse applications in forensic science, failure analysis, quality control and materials research. The clinical result of these can be enhanced by carrying out implant retrieval analyses.

This study aimed to use SEM and EDS to conduct surface and elemental investigations of two distinct retrieved titanium miniscrew implants which are most commonly used in local population with the primary objective being investigation of how the tiny implant behaved when it came in contact with food, mouth fluid, surrounding tissues, and bone. This study aimed to analyze alterations in the surface morphology, elemental composition and flexural strength of 2 brands of miniscrew implants (Favanchor and Orlus) before and after clinical use.

MATERILAS AND METHODS

This in-vitro study utilized convenience sampling to select 20 samples of miniscrew implants, which were used for orthodontic treatment in patients treated at the Department of Orthodontics and Dentofacial Orthopaedics, Dr. D.Y Patil Dental College and Hospital, Pimpri, Pune. This study was approved by the Institutional Ethical Review Committee (Ref: DPU/806/9/2023). A waiver of consent was granted in accordance with the ICMR Ethical Guidelines 2017.

Inclusion criteria for the selection of the patients from whom the retrieved mini-screw implants used for testing was those who needed temporary anchorage devices during corrective orthodontic treatment for maxillary space closure for a minimum of 6 months. The exclusion criteria consisted of failed implants, patients with syndromes or autoimmune diseases, those who were pregnant or lactating, and individuals on medications such as antibiotics, antihistamines, cortisone, hormones, or any other medication that may interfere with the inflammatory process or adversely affect the periodontium.

Self-drilling orthodontic miniscrew screw implants of two commercially available brands were used in this study. They were divided into two groups: Group I: Favanchor, S.H.Pitkar Ortho tools Pvt Ltd, Pune (10 samples: 1 Favanchor miniscrew in as received state which acted as the control and 9 Favanchor miniscrews which were retrieved after their use in patients undergoing orthodontic treatment which formed the test group) and Group II: Orlus, Ortholution, Korea (10 samples: 1 Orlus miniscrew in as received state which

acted as the control and 9 Orlus miniscrews which were retrieved after their use in patients undergoing orthodontic treatment which formed the test group).

Thus, a total of 20 mini-screw implants were used. One miniscrew implant from each manufacturer was analysed with a scanning electron microscope in the as received state, and 18 miniscrew implants were retrieved ones which were retrieved after their use in patients. These mini screw implants were used in patients following consistent guidelines mentioned below.

A standardized clinical protocol was employed for the placement and utilization of all miniscrew implants, with a single operator executing the procedure in accordance with established departmental guidelines. The miniscrew implants were inserted and removed by the same operator, who followed the placement protocol recommended for a self-drilling miniscrew - implant. The miniscrew implants were retrieved after completion of their role in the orthodontic treatment of each patient. After removal, each retrieved miniscrew implant was washed gently and stored by completely immersing in fresh deionized water in an autoclaved glass vial that was duly labelled.

Thus the 20 samples were subjected to the following analysis:

1. Energy dispersive x-ray microanalysis (EDX) using Bruker XFlash 6130
2. Scanning electron microscopy (SEM) using FEI Nova NanoSEM 450
3. Flexural strength analysis was by using Universal Testing Machine (ACME Engineers, India. Model No. UNITEST-10).

One miniscrew implant from each manufacturer were subjected to the tests (SEM, EDX and Flexural strength analysis) before clinical use and 18 miniscrew implants (9 each from the two brands being tested) were inserted into patients. The miniscrew implants were placed in maxilla and were immediately loaded as per protocols used in the department according to the treatment plan. Implants were retrieved from the oral cavity after their role is completed in orthodontic treatment of each patient. Implants were gently washed and stored completely immersed in fresh deionized water in an auto-claved glass vial duly labelled. The miniscrew implants were examined using SEM for evaluation of their surface topography and digital images were acquired at different magnifications. Composition of elements adsorbed on the miniscrew implant surface were evaluated with an EDX coupled to the SEM. Flexural strength were determined by using universal testing machine. Results from the three tests were analysed and subjected to intra-group and inter- group comparisons to assess the miniscrew implants' alterations after clinical use and contact with the oral environment. Analyses by energy dispersive x-ray microanalysis (EDX) and scanning electron microscopy (SEM) were done at 4 zones of each MSI: head, neck, body, and tip (Fig 1).

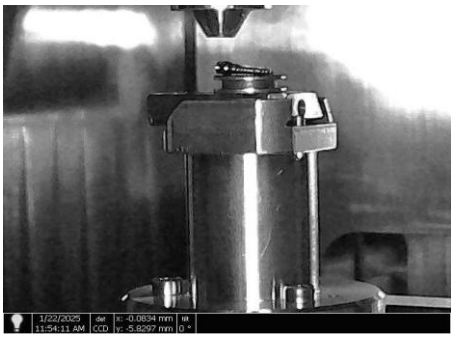


Figure 1. Showing mini-screw implants mounted on aluminium supporting discs using double carbon sided tape under the microscope.

SEM of all the samples was done under **FEI Nova NanoSEM 450** at an operating voltage of 15 kV with the miniscrew screws mounted on aluminium supporting discs using double carbon sided tape. Alterations of the surface such as crevice corrosion, corrosion surface damages, dullness, cracks, craters, fractures and blunting, were looked for in each zone.

Surface alterations categorised in this study were as follows:

- Craters: formation of cavities or holes
- Cracks: lines on the surface indicating splitting without separation
- Fracture: cracking or breaking of the material
- Dullness: loss of glossiness and finish
- Blunting: loss of sharp edges of the threads or the tip
- Corrosion Damage: crevice-like geometric patterns, pitting and fretting.

Each zone of every sample was scored with presence (1) or absence (0) for each of the attributes mentioned above. The maximum score for each zone (i.e. head, neck, body and tip) can be 9 for retrieved group and 1 for the control group for the presence of the attribute. The minimum score for each zone can be 0 for both the groups for the absence of the attribute.

After initial scanning of each zone, images of the damaged features or areas were captured. Multiple images of each zone of the MSI were taken; the number of images varied for each zone (about 2-4 images per zone of each MSI). Digital images were obtained at various magnifications (20-500 times) in an incremental manner.

An EDX detector (Bruker XFlash 6130) was used to investigate their elemental composition with an x-ray microanalysis detector. The quantitative analysis of the percentage of weight concentration of the probed elements was performed with an INCA micro-analyzer. The technical limitations of EDX spectroscopy in the electron microscope precluded the quantitative analysis of carbon in this investigation. Elements with lower atomic masses such as carbon (atomic number 6 and lower) are difficult to distinguish from each other using EDX. The carbon x-rays have low energy and are easily absorbed by the x-ray detector windows. Furthermore, there can be a significant carbon background signal because of hydrocarbon contamination. Hydrocarbons from the chamber surfaces, vacuum pumps, and sample surface migrate and react with the electron beam to form a black spot that is rich in carbon.

For each brand, 1 as received and 9 retrieved specimens were tested with a Universal Testing Machine (ACME Engineers, India. Model No. UNITEST- 10). Each miniscrew implant was blocked in the lower jaw of the machine with the head end of the miniscrew free. (Fig 2). The portion of the miniscrew between the endoosseous thread and transmucosal collar was then exposed to a tangential load with a 0.5mm/min crosshead speed. Bending force was measured at 0.1 mm and 0.2 mm deflections.

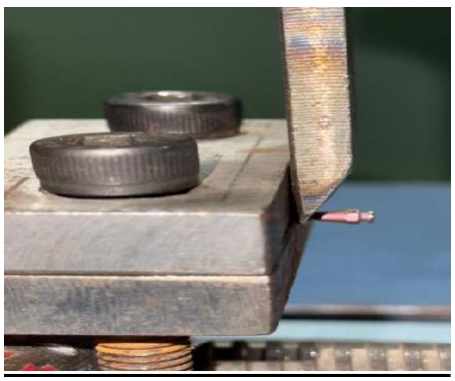


Figure 2. Showing miniscrew implants blocked in lower jaw of universal testing machine

RESULTS

The results of the study, as shown in Figure 3, 4, present scanning electron micrographs of the head, neck, body, and tip of Favanchor and Orlus miniscrew implants, highlighting various surface alterations after clinical use.

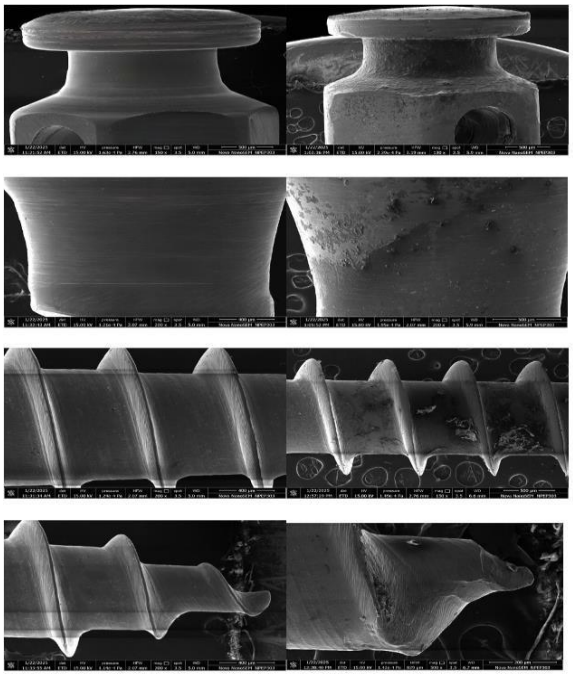


Figure 3. Scanning electron microscopic images of head, neck, body,tip of received FAVANCHOR titanium implants(left) and retrieved FAVANCHOR titanium(right) implants showing cracks, dullness corrosion, and blunting

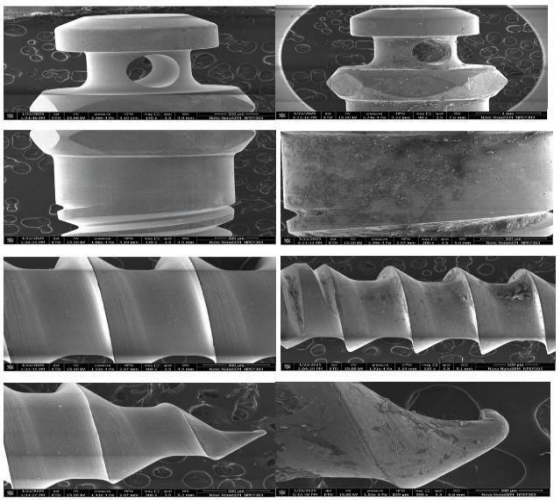


Figure 4.Scanning electron microscopic images of head, neck, body,tip of received ORLUS titanium implants(left) and retrieved ORLUS titanium(right) implants showing cracks, dullness corrosion, and blunting

Both Favanchor and Orlus implants exhibit dullness, cracks, and craters across all zones, with Favanchor implants showing additional fractures and blunting, especially in the body and tip regions. Statistical analysis, performed using SPSS version 20, involved non-parametric tests, including the Kruskal-Wallis test to compare elemental composition (EDX data), post-hoc Wilcoxon rank-sum tests with Bonferroni correction, chi-square tests for SEM comparisons, and unpaired t-tests for flexural strength. The findings are summarized in Table 1, which compares surface changes between Favanchor and Orlus implants, showing corrosion, dullness, cracks, craters, fractures, and bluntness, with Favanchor implants exhibiting more damage in the body and tip.

Table 1. Surface Changes on Mini-Implants (Favanchor vs. Orlus)

Implant Type	Corrosion	Dullness	Cracks	Craters	Fracture	Bluntness
Favanchor	Yes (Head, Neck, Body, Tip)	Yes (Head, Neck, Body, Tip)	More in Body & Tip	More in Body & Tip	Higher in Body & Tip	Similar in Body & Tip
Orlus	Yes (Head, Neck, Body, Tip)	Yes (Head, Neck, Body, Tip)	Less frequent	Less frequent	Higher in Body	Similar in Body & Tip

Table 2 details the elemental composition comparison, showing significant differences in titanium, aluminum, iron, and oxygen content between the two implant brands.

Table 2. Elemental Composition Comparison (Favanchor vs. Orlus)

Element	Head	Neck	Body	Tip	P- value for comparison within zones (Kruskal-Wallis test)
Titanium					
Favanchor- R	60.06	61.49	58.64	40.61	0.00006 *
Favanchor- C	62.32	70.6	73.12	44.71	
Orlus – R	57.92	69.03	61.47	53.30	< 0.00001*
Orlus - C	64	76.17	71.78	51.95	
Aluminium					
Favanchor- R	5.6	3.96	3.15	12.56	< 0.00001 *
Favanchor- C	5.58	3.79	3.4	14.7	
Orlus – R	3.57	3.40	2.99	1.41	0.00001 *
Orlus - C	3.49	4.13	4.92	1.17	
Vanadium					
Favanchor- R	1.62	1.63	1.86	1.56	0.13588
Favanchor- C	2.65	1.55	2.37	1.05	
Orlus – R	1.32	2.01	2.27	1.40	< 0.00001 *

Orlus - C	0.6	0.56	0.2	0.32	
Nitrogen					
Favanchor- R	0.28	0	0	0	0.80779
Favanchor- C	0.21	0	0	0	
Orlus – R	0	0	0	0	-
Orlus - C	0	0	0	0	
Silicon					
Favanchor- R	0	0	0	0	-
Favanchor- C	0	0.15	0	0	
Orlus – R	0	0	0	0	-
Orlus - C	0	0	0	0	
Chromium					
Favanchor- R	0	0	0	0	-
Favanchor- C	0	0	0	0	
Orlus – R	0	0	0	0	-
Orlus - C	0	0	0	0	
Copper					
Favanchor- R	0	0	0	0	-
Favanchor- C	0	0	0	0.34	
Orlus – R	0	0	0	0	-
Orlus - C	0	0	0	0	
Chlorine					
Favanchor- R	0	0	0	0	-
Favanchor- C	0	0	0	0.54	
Orlus – R	0	0.18	0	0	0.97032
Orlus – C	0	0	0	0	
Phosphorous					
Orlus – R	0	0.05	0.05	0	0.83558
Orlus – C	0	0	0	0	
Sodium					
Orlus – R	0	0	0.10	0	0.80779
Orlus – C	0	0	0	0	

Table 3 compares the flexural strength of the implants, demonstrating significant differences between control and retrieved groups for both implant types.

Table 3. Flexural Strength Comparison (Favanchor vs. Orlus)

Implant Type	0.1mm Deflection	0.2mm Deflection	P-value
Favanchor	51.33 (R)	98.56 (R)	<0.05 (0.1mm), <0.05 (0.2mm)
Orlus	71.56 (R)	156.00 (R)	<0.05 (0.1mm), <0.05 (0.2mm)

Figures 5 and 6 present representative EDX plots of as-received and retrieved Orlus miniscrews, providing insight into the elemental composition of the implants before and after clinical exposure. Similarly, Figures 7 and 8 show the corresponding EDX plots for Favanchor implants, comparing the as-received and retrieved states. These figures reveal significant changes in elemental content, particularly in oxygen, iron, and calcium levels. Figures 9 to 11 graphically depict the flexural strength of the miniscrews at 0.1mm and 0.2mm deflections. Figure 9 compares the mean flexural strength of Favanchor implants in their as-received and retrieved states, while Figure 10 does the same for Orlus implants. Figure 11 further contrasts the flexural strength between the retrieved Favanchor and Orlus implants, showing a significant decrease in strength after clinical use. These figures collectively underscore the mechanical degradation and surface alterations in both types of miniscrews, reflecting their interactions with the oral environment and their impact on clinical performance.

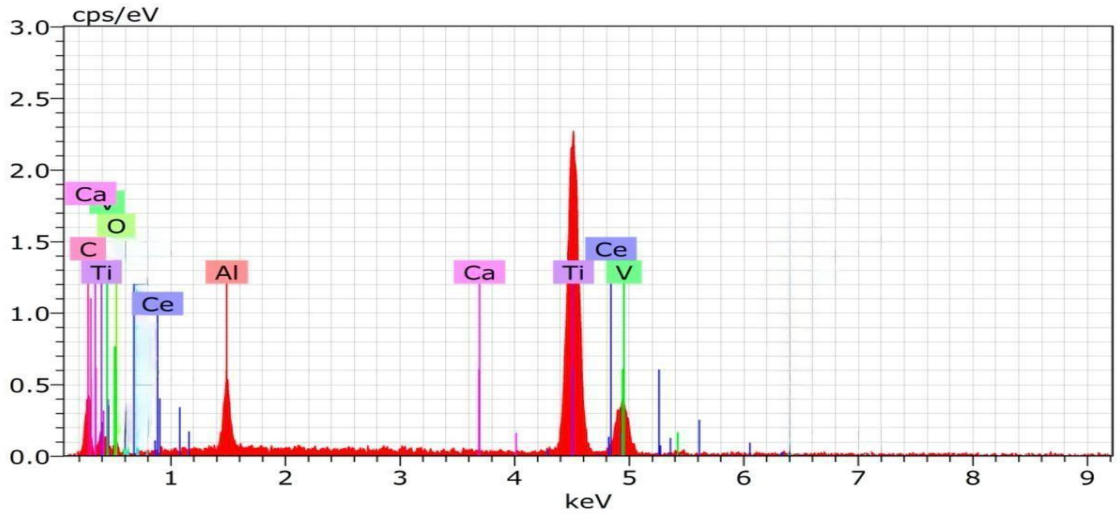


Figure 5. Representative graph of EDX plot of as-received Orlus mini-implant

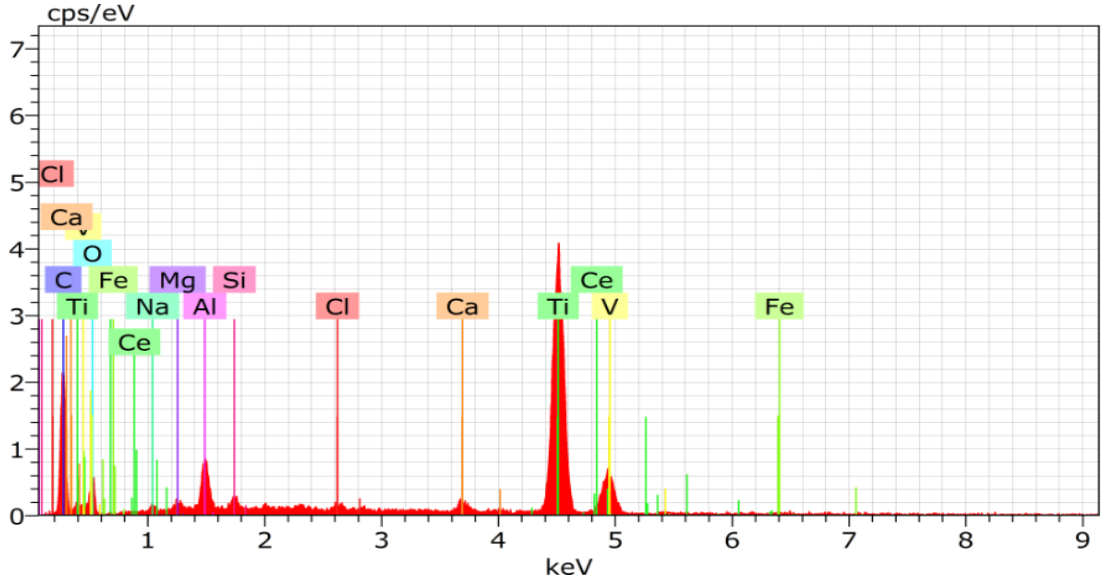


Figure 6. Representative graph of EDX plot of retrieved Orlus mini-implant

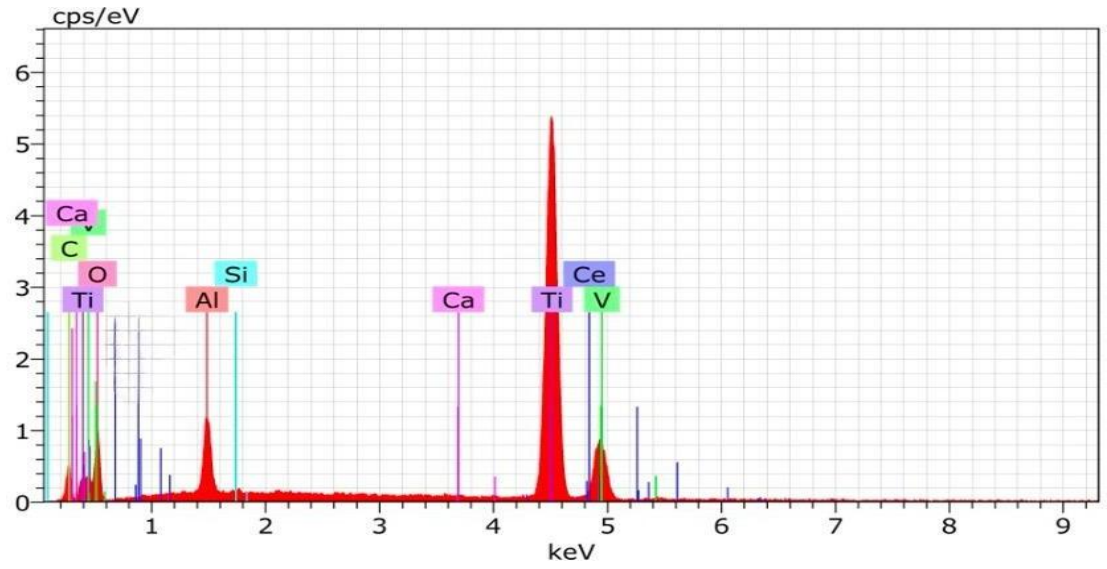


Figure 7. Representative graph of EDX plot of as-received Favanchor mini-implant

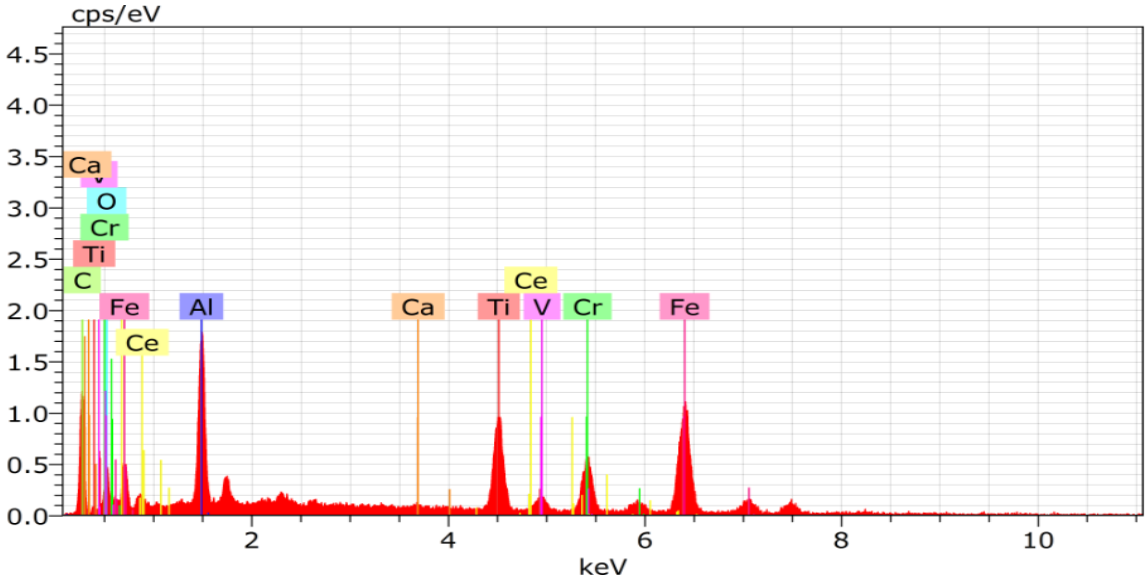


Figure 8. Representative graph of EDX plot of retrieved FAVANCHOR mini-implant

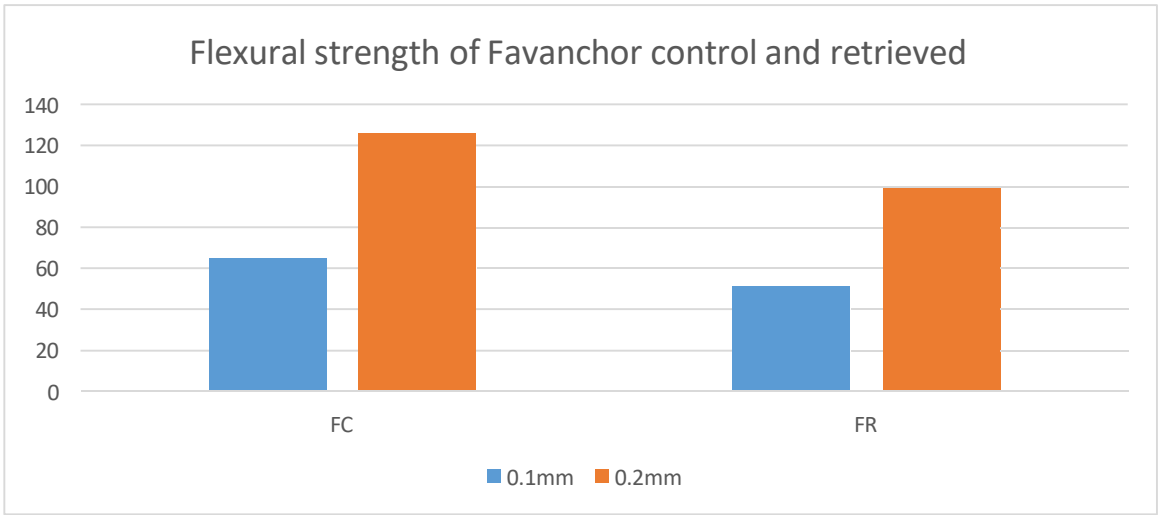


Figure 9. Graphical representation of mean flexural strength at 0.1mm in in Favanchor control and Favanchor retrieved at 0.1mm and 0.2 mm deflection

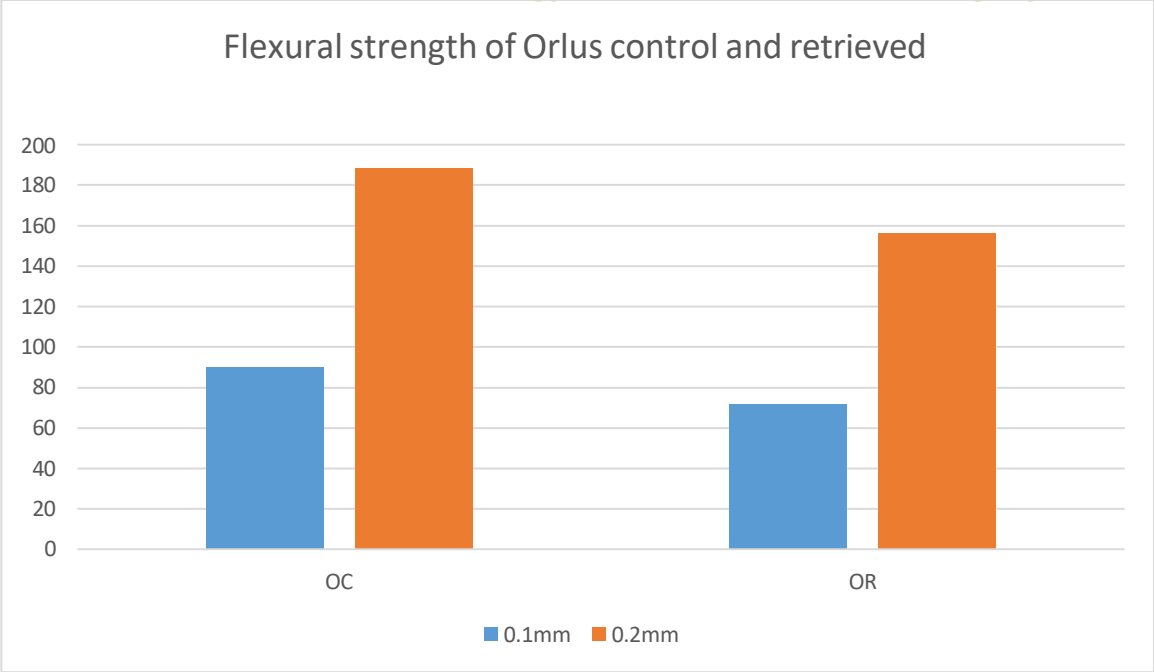


Figure 10. graphical representation of mean flexural strength at 0.1mm in in Orlus control and Orlus retrieved at 0.1mm and 0.2 mm deflection

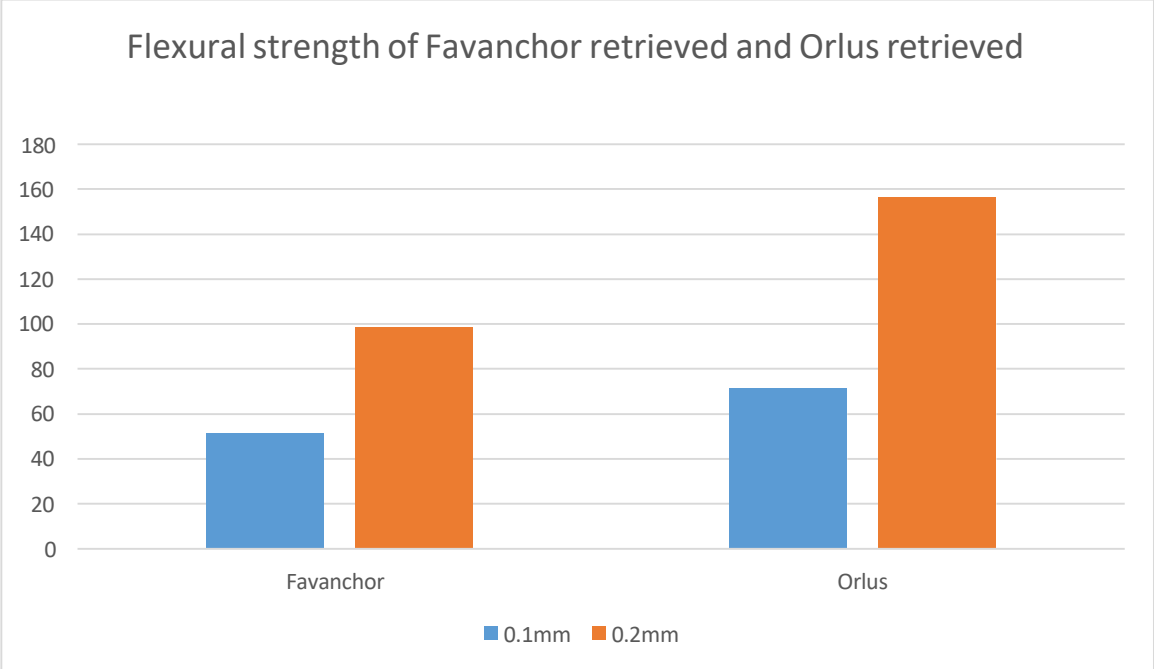


Figure 11. Graphical representation of mean flexural strength at 0.1mm in Favanchor retrieved and Orlus retrieved at 0.1mm and 0.2 mm deflection

Data analysis was performed using SPSS version 20 (IBM, Armonk, NY). Non- parametric tests were employed due to the nature of the data. The Kruskal-Wallis test was used to compare elemental composition (EDX data), followed by post-hoc Wilcoxon rank-sum tests with Bonferroni correction for pairwise comparisons. The chi-square test was utilized for intergroup and intragroup comparisons of SEM results. unpaired t-tests were conducted to compare flexural strength between and within groups. Statistical significance was set at $P < 0.05$.

Iron had a mean value of 1.23 in the head region of retrieved Favanchor miniscrew implant, 0.28 in the neck region, 0.05 in the body region and 2.08 in the tip region which on comparison with the control Favanchor miniscrew implant {Head(H) = 0.51, Neck (N)=0, Body (B)=0, Tip (T)=0} was statistically significant ($p<0.00001$). Similarly on comparison of the retrieved

Orlus miniscrew implant H=0.57, N=0.12, B=0.2, T=0.06 and in control was H=0.57, N=0.35, B=0.53, T=0.14 was statistically significant (p=0.00053).

Calcium had a mean value of 0.2 in the head region of retrieved Favanchor miniscrew implant, 0.18 in the neck region, 0.3 in the body region and 0.35 in the tip region which on comparison with the control Favanchor miniscrew implant {Head(H) = 0.15, Neck (N)=0.06, Body (B)=0.03, Tip (T)=0.35} was statistically not significant. Similarly on comparison of the retrieved Orlus miniscrew implant H=0.25, N=0.54, B=0.61, T=0.13 and in control was H=0.17, N=0, B=0.1, T=0.01 was statistically significant (p<0.00001).

Cerium had a mean value of 0.35 in the head region of retrieved Favanchor miniscrew implant 0.26 in the neck region, 0.38 in the body region and 0.04 in the tip region which on comparison with the control Favanchor miniscrew implant {Head(H) = 0, Neck (N)=0, Body (B)=0, Tip (T)=0} was statistically significant (p=0.03638). Similarly on comparison of the retrieved Orlus miniscrew implant H=0.09, N=0.23 B=0.21, T=0.06 and in control was H=0.6, N=0.56, B=0.2, T=0.32 was statistically not significant.

DISCUSSION

Our study discusses the surface changes seen on MSIs post retrieval and is compared to the implant in as received state. The emergence of retrieval analysis in biomedical material science provides valuable insights on the in vivo performance and durability of biomaterials within their designated environments.

Our study showed substantial changes in the surface topography of MSIs and the presence of impurities on their surfaces thought to result from contact with oral tissues, biological fluids, and other substances. Changes were seen in four different zones of implant i.e. head, neck, body and tip all thought to be unique due to different environmental exposures in the body. Our findings reveal substantial alterations in the surface topography of retrieved metal-supported implants (MSIs), characterized by the presence of cracks, craters, fractures, blunting, and the accumulation of precipitated impurities on the implant surfaces. These surface modifications are attributed to complex interactions between the implant and various biological factors, including oral tissues, bodily fluids, hematological components, exudates, saliva, and dietary elements [16].

A comprehensive analysis of retrieved Miniscrew Implants (MSIs) revealed a significant degradation of surface characteristics, manifesting as a loss of gloss and surface finish, resulting in a dull appearance uniformly across all examined zones. It is hypothesized that the insertion and removal processes of the miniscrews contributed substantially to surface wear, leading to a deterioration of their morphological integrity. Furthermore, thread blunting and tip dulling, characterized by a reduction in thread sharpness and tip acuity compared to new screws, were also attributed to wear-induced degradation during the insertion and removal process. Occasional fractures observed at the peripheral border or tip of the thread were ascribed to localized reductions in mechanical strength, rendering these regions more susceptible to failure.

Significant corrosion damage and crater formation were observed on the surface of retrieved MSIs, particularly in those that had been in place for extended periods which allowed prolonged exposure to oral tissues and biological fluids. Conversely, cracks and fractures were predominantly localized to the thread and tip regions, likely owing to the reduced material thickness in these areas, which rendered them more susceptible to mechanical failure and stress concentration. These results are in accordance with the study by Natarajan and Rao [17].

The primary constituent elements of the retrieved Miniscrew Implants (MSIs) were obscured due to the adsorption of other elements from the surrounding biological environment onto the surface. Since Energy Dispersive X-ray (EDX) spectroscopy provides elemental data in terms of weight percentages, a noticeable reduction in the concentration of the parent (titanium, aluminium and vanadium) materials was observed when comparing the metal components of the titanium implants before and after recovery. This decrease in elemental content was attributed to the presence of elements such as oxygen, nitrogen, calcium, phosphorus, iron, sodium, chlorine, magnesium, and potassium. These substances, adsorbed from the surrounding biological environment, effectively masked the primary components on the implant surface.

The formation of a titanium oxide passive layer on the surface of titanium, resulting from its interaction with oxygen, is a well-documented phenomenon. This suggests that the observed increase in oxygen concentration may have contributed to the development or enhancement of the passive oxide layer on the retrieved MSIs.

The presence of calcium on the implant surface is primarily attributed to the interaction between the implant and blood, wherein proteinaceous substances are adsorbed, followed by mineralization through the precipitation of calcium and phosphorus ions. Furthermore, the adherence of bone particles to the miniscrew implant surface is a direct consequence of its contact with the surrounding alveolar bone, resulting in

the transfer and deposition of bone fragments onto the implant surface. This phenomenon highlights the implant's integration with the host bone tissue. The retrieved group exhibited higher levels of calcium, particularly in the body region, as indicated by the weight percentage. Calcium levels were present in both retrieved Favanchor miniscrew implant and retrieved Orlus miniscrew implants in the head region. In the neck region, calcium levels were present in both retrieved Favanchor and retrieved Orlus miniscrew implants, with higher levels in retrieved Orlus. Calcium levels were also present in both retrieved Favanchor and retrieved Orlus miniscrew implants in the body region, with higher levels in retrieved Orlus miniscrew implants. In the tip region, calcium levels were present in both retrieved Favanchor and retrieved Orlus miniscrew implants, with varying levels between the two.

Iron levels were higher in the retrieved Favanchor miniscrew implant compared to the control in the head region. In the neck region, iron levels were present in the retrieved Favanchor miniscrew implant, whereas they were absent in the control. The body region of the retrieved Favanchor miniscrew implant had iron levels, whereas the control had none. In the tip region, iron levels were higher in the retrieved Favanchor miniscrew implant compared to the control. The higher proportion of iron observed in the retrieved miniscrews, as indicated by the weight percentage data was attributed to their contact with blood. When compared to retrieved implants of both the brands, it was seen more in Favanchor miniscrew implant than Orlus miniscrew implant.

The retrieved miniscrew Implants (MSIs) showed a higher concentration of cerium in the head and neck regions compared to the body and tip regions. It was seen more in Favanchor miniscrew implant than Orlus miniscrew implants. Cerium, a element possessing antimicrobial properties, is a constituent of select mouthwashes and is also present in certain food items, such as tubers cultivated in cerium-rich soils and water sources. In the present study, the detection of cerium on the head and neck regions of the retrieved miniscrew implants (MSIs) was attributed to exposure to cerium-containing mouthwashes and ingestion of cerium-rich food sources, highlighting the potential for environmental and lifestyle factors to influence the surface composition of implanted devices. [18,19] These findings corroborate with the study done by Patil et al. [20]

Additionally, trace quantities of various elements, including chromium, fluorine, sodium, chlorine, magnesium, and potassium, were identified. These elements are ubiquitous in everyday substances, such as food, beverages, mouthwashes, toothpaste, and drinking water, suggesting that their presence on the implant surface is likely due to environmental exposure and routine oral hygiene practices.

The outermost atomic layers of miniscrew Implants (MSIs) are crucial areas involved in the biochemical interactions at the implant-tissue interface. An escalation in corrosion severity can compromise the mechanical and biochemical properties of titanium-based mini-implants, potentially leading to diminished structural integrity and functionality. Furthermore, the release of elemental constituents from the mini-implants can modulate the inflammatory response, increasing the likelihood of adverse complications or implant failure. These findings underscore the importance of optimizing the corrosion resistance and biocompatibility of titanium-based mini-implants to ensure their safe and effective use in clinical applications. These regions significantly impact the need for high levels of standardization and surface control during MSI production.

This study had several limitations such as a small sample size, no effort made for categorization into age groups based on their growth status (growing or nongrowing), which could possibly influence bone density thus leading to further alterations in implant topography. Also the selected implants were not subject to pre-implantation SEM and EDX analysis suggesting that their surface characteristics and chemical composition were assumed to be consistent with the manufacturer's specifications, which were presumably established through proper manufacturing protocols and quality control measures. This assumption is based on the understanding that the implants were fabricated using standardized methods and materials, and that the manufacturer's quality control processes ensured that the implants met the required standards for biocompatibility and performance.

CONCLUSION

In conclusion, the implants in the as-received groups (both Favanchor and Orlus) showed no structural damage, with flexural strength being higher for the as-received Orlus miniscrew implants compared to Favanchor miniscrews. However, the retrieved orthodontic miniscrew implants (MSIs) displayed significant morphological changes, including surface dullness, thread and tip blunting, corrosion, crater formation, and occasional fractures. A comparison between retrieved and new MSIs revealed notable differences in surface elemental composition, with the retrieved MSIs containing additional elements such as oxygen, carbon, iron, calcium, cerium, fluoride, and sodium. Calcium was most prominently observed in the body region, with higher levels seen in the Orlus

miniscrews. Iron deposits were present in the body and tip regions of both implant types, with Favanchor showing more iron content. Cerium was found in the head and neck regions, more prominently in the Favanchor implants. Furthermore, there was a decrease in the flexural strength of both retrieved miniscrew implant brands. These findings underline the potential limitations in reusing retrieved miniscrew implants due to structural and elemental alterations.

DECLARATIONS

No funding was received from any financially supporting body, and there was no associated grant number. No funder was involved in manuscript writing, editing approval, or decision to publish.

Consent for publication

Informed consent was obtained from every participant for documentation and examination.

Competing interests

The authors declare no competing interests.

Ethical approval

Ethical approval was granted by the Institutional Human Ethical Committee

Informed patient consent

All patients' clinical records were obtained with informed consent.

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COMPARISON BETWEEN STANDARD PROTOCOL AND GUIDED BIOFILM THERAPY IN PROFESSIONAL ORAL HYGIENE

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ABSTRACT

Background: Professional oral hygiene is pivotal in preventing periodontal disease by eliminating supragingival and subgingival biofilm and calculus. While traditional methods are effective, they may induce discomfort and tissue trauma. Guided Biofilm Therapy (GBT) is a minimally invasive protocol designed to optimize biofilm removal while improving patient comfort and operator ergonomics.

Aim: This study compares the Standard Protocol and GBT in terms of clinical efficacy, patient-reported comfort, treatment duration, and operator experience

Materials and Methods: The Present in vitro study utilized six subjects' blood and was centrifugated. T-PRF clots were prepared, and TI/ NE gel extracts were injected and compared with T-PRF alone to check the release of Vit C, Az, and IGF 1 at 6hours, 72 hours (3rd), 7th, and 14th day. For statistical analysis, a paired t-test and ANOVA were performed. P value <0.05 was considered statistically significant.

Results: Over 98% of patients treated with GBT reported reduced or absent pain compared to traditional methods. GBT reduced treatment time by approximately 15% and was associated with improved plaque control, reduced bleeding, and lower probing depths. Operators reported reduced fatigue and improved ergonomics with GBT instrumentation.

Conclusions: GBT offers a clinically effective and patient-friendly alternative to conventional prophylaxis, combining enhanced comfort with efficient biofilm management. These findings support the integration of GBT into routine oral hygiene practice to optimize clinical outcomes and patient compliance.

Key Words: GBT, scaling, professional hygiene

1. INTRODUCTION

Periodontal disease is a multifactorial chronic inflammatory condition initiated by dysbiotic biofilm and influenced by systemic and local risk factors. Its effective management requires mechanical disruption of biofilm and patient adherence to preventive protocols. Traditional supragingival prophylaxis techniques—primarily manual or ultrasonic scaling—are often associated with discomfort, abrasiveness and variability in operator technique may limit their long-term efficacy and patient acceptance.[1-4] Guided Biofilm Therapy (GBT), developed by EMS, represents a structured, evidence-informed protocol to address these limitations. It integrates biofilm disclosing, air polishing with low-abrasive erythritol or glycine powders, selective use of ultrasonic devices, and patient education, all within a standardized eight-step workflow (1,5-7). This patient-friendly protocol emphasizes biofilm visualization and atraumatic debridement as central elements of modern periodontal maintenance. GBT is supported by recent literature demonstrating improvements in clinical outcomes and patient compliance. Clinical trials have shown reductions in bleeding on probing, plaque index, and gingival inflammation in patients treated with GBT compared to traditional methods[9-10]. Additionally, increased comfort and reduced procedural pain have been consistently reported, reinforcing its role in enhancing patient experience during hygiene sessions.

This study aims to assess the subjective response of patients undergoing GBT, with specific attention to comfort, pain, motivation, and overall satisfaction, using patient-reported outcome measures (PROMs).

2. MATERILAS AND METHODS

The findings aim to support the integration of GBT as a standard approach in prophylaxis, particularly for patients undergoing long-term periodontal maintenance.[11-12]



Figure 1. GBT technique

This comparative observational study enrolled adult patients in a professional oral hygiene recall program. Eligibility required good systemic health, the absence of periodontal conditions requiring surgical intervention, and informed consent. Patients were divided into two groups based on the hygiene protocol: the Standard Protocol group and the Guided Biofilm Therapy (GBT) group. The Standard Protocol included manual and ultrasonic scaling followed by rotary polishing using abrasive paste. The GBT protocol, in contrast, consisted of an eight-step sequence: medical history review, disclosing agent application for biofilm visualization, personalized hygiene instruction, air-polishing with erythritol powder via AirFlow®, subgingival instrumentation with PerioFlow® and Piezon® devices as needed, and final polishing. Each treatment was performed by experienced dental hygienists trained in both methods. Patient-reported experience was collected via a structured questionnaire assessing pain, comfort, noise level, and overall satisfaction using a 10-point Visual Analogue Scale (VAS). The operator recorded the treatment time. Ergonomic feedback was gathered through an operator-completed Likert scale regarding fatigue, posture, and instrument handling.Data were analyzed descriptively. Patient outcomes were compared qualitatively between groups, while ergonomic impact and efficiency were assessed from the operator's perspective.





Figure 2. Treatment with Guided Biofilm Therapy after plaque relief using colored relevant agents

3. RESULTS

The results of this observational study highlight a clear advantage of Guided Biofilm Therapy (GBT) over the Standard Protocol across all measured domains. Patients treated with GBT reported significantly lower pain levels, with an average VAS score of 1.8 (±0.6), compared to 5.8 (±0.9) in the control group. This substantial difference underscores the more atraumatic nature of the GBT approach.

In terms of perceived comfort during the procedure, GBT again outperformed the standard method. Patients rated their experience as markedly more comfortable, assigning an average score of 9.2 (±0.7) versus 6.5 (±1.1) for those treated with conventional scaling techniques. Similarly, noise sensitivity ratings favored GBT, with participants assigning it a mean score of 8.7 (±0.5), compared to 6.2 (±0.9) for the Standard Protocol, suggesting improved tolerability of the instrumentation used in GBT.

Overall treatment satisfaction was also higher in the GBT group, with an average score of 9.5 (±0.4) compared to 6.7 (±1.0) in the control group. Notably, a large majority—92%—of patients clearly preferred GBT and indicated they would choose it again for future sessions. This suggests that the subjective patient experience strongly favors the GBT approach.

Despite its structured workflow, the GBT protocol did not prolong treatment time. On the contrary, appointments were completed approximately 15% faster than with the standard protocol. This time efficiency likely results from the streamlined plaque removal process enabled by the AirFlow® system, which minimizes the need for prolonged mechanical instrumentation.

Lastly, the clinicians involved reported decreased physical strain and hand fatigue during GBT procedures.

4. DISCUSSION

The data showed that patients in the GBT group experienced significantly more favorable outcomes compared to the Standard Protocol group across multiple parameters. Pain levels reported during GBT were markedly lower, with a mean VAS pain score of 1.8, compared to 5.8 in the traditional group. The GBT

approach also received higher ratings for comfort (mean 9.2 vs 6.5), reduced perceived noise (8.7 vs 6.2), and greater overall satisfaction (9.5 vs 6.7) (Figure 3).

Patient motivation appeared enhanced by the disclosing phase of GBT, with many respondents noting that biofilm visualization helped them understand oral hygiene priorities more clearly. In addition, 92% of patients preferred GBT over traditional scaling methods (Figure 4), citing improved tolerability and reduced post-operative discomfort.[13-15]

Despite a more structured and multi-step approach, Treatment time was reduced by 15% in the GBT group. This efficiency may be attributable to the faster biofilm removal capacity of the AirFlow® device compared to manual instrumentation.[16-20]

Ergonomically, hygienists consistently rated GBT as less physically demanding. The design of the AirFlow® handpiece and the minimized need for repetitive manual scaling translated into reduced musculoskeletal strain, improved posture maintenance, and decreased session fatigue. Operator satisfaction aligns with existing literature supporting the ergonomic benefits of air-polishing systems in dental hygiene settings.

These findings complement prior clinical studies demonstrating that GBT significantly reduces bleeding on probing and gingival inflammation, with fewer adverse tissue effects due to the gentle action of erythritol and glycine powders[21,22]. Although this study was observational in nature, the consistency in subjective measures supports GBT's implementation in maintenance care. [23-24]

The data collected in this observational study support the clinical and experiential superiority of Guided Biofilm Therapy (GBT) over the Standard Protocol for professional oral hygiene. Across all measured domains—pain perception, comfort, noise sensitivity, satisfaction, treatment efficiency, and operator ergonomics—GBT consistently yielded more favorable outcomes.

Patient-Reported Outcomes

Pain was significantly reduced in the GBT group, with a mean VAS score of 1.8, compared to 5.8 in the Standard Protocol group. This aligns with prior findings demonstrating that air-polishing with erythritol powder induces less soft tissue trauma than traditional ultrasonic scaling (16,19). Similarly, patients rated GBT higher in comfort (9.2 vs 6.5), noise tolerance (8.7 vs 6.2), and overall satisfaction (9.5 vs 6.7). These values reflect the GBT workflow's less invasive, more predictable, and patient-centric nature.

An additional strength of GBT lies in its visual component. The disclosing phase enhanced patient

motivation by providing immediate biofeedback on biofilm presence. This educational aspect fosters behavioral reinforcement, as previously noted by Furrer et al. [19], who reported improved home care adherence in patients exposed to biofilm visualization. Furthermore, 92% of patients expressed a preference for GBT over conventional scaling, not only due to reduced procedural discomfort but also because of a lower incidence of post-treatment sensitivity and bleeding. This subjective preference supports the notion that PROMs are critical in determining patient compliance and return rates for maintenance therapy [13–15].

Clinical Efficiency and Operator Experience

Despite its structured eight-step design, GBT showed a 15% reduction in treatment time, likely due to the enhanced efficiency of air-polishing devices in biofilm disruption. The AirFlow® system, in particular, has been demonstrated to remove supragingival and subgingival plaque more uniformly and rapidly than manual methods [16,20].

From an ergonomic perspective, GBT reduced perceived fatigue among hygienists. Thanks to the lightweight and ergonomic design of the GBT instrumentation, operators reported improved posture and less hand strain. These findings are consistent with literature suggesting that repetitive manual scaling contributes to musculoskeletal disorders among dental professionals and that air-polishing can mitigate this risk [21].

Comparison with Literature

The present results corroborate previous clinical studies showing GBT's effectiveness in reducing bleeding on probing and gingival inflammation, likely due to the anti-inflammatory effects of erythritol and the atraumatic application technique [11,17,21]. Additionally, GBT may reduce the cumulative trauma associated with repeated instrumentation, especially important in patients with exposed root surfaces, restorations, or dental implants [15,17,23]. In the broader context of oral hygiene protocols, the adjunctive use of chemical agents plays a fundamental role in enhancing the effectiveness of mechanical plaque removal. Among these, stannous fluoride has demonstrated significant potential in terms of enamel protection, antimicrobial activity, and reduction of gingival inflammation. A systematic review has recently summarized its multifactorial benefits, supporting its integration within routine oral hygiene regimens, particularly for patients undergoing scaling procedures or at high risk for caries and erosion. Furthermore, it should be acknowledged that the long- term success of periodontal and peri-implant maintenance protocols—particularly in patients rehabilitated with implant-supported prostheses—relies not only on professional debridement and oral hygiene measures, but also on patient satisfaction, compliance, and prosthetic stability.

Recent clinical studies have investigated aspects such as the longevity of implant- retained overdentures and the application of fully digital workflows in implant-supported restorations, which can significantly influence plaque control and access to hygiene procedures in the long term. Moreover, the prevention and management of enamel demineralization phenomena, such as white spot lesions, represent a critical endpoint in the continuum of care related to oral hygiene. These lesions often result from inadequate plaque control and are frequently observed in patients undergoing orthodontic treatment or those with poor compliance with oral hygiene instructions. Recent systematic reviews have emphasized the importance of early detection and the integration of non-invasive therapeutic strategies as part of a comprehensive hygiene protocol. In the broader context of emerging biofilm control strategies, recent investigations have highlighted the potential of adjunctive therapies such as ozonated olive oil in modulating oral microbiota, reducing inflammation, and enhancing mucosal healing. These approaches align with minimally invasive, patient- centered dentistry principles and may complement mechanical debridement methods in selected clinical scenarios [24-29].

While the study's observational nature precludes definitive causal inferences, aligning subjective outcomes with GBT's mechanistic advantages lends credibility to its implementation in maintenance protocols.

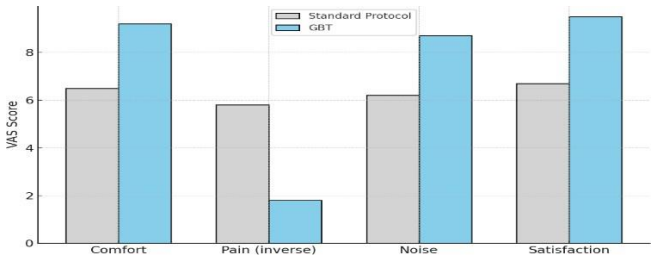


Figure 3. Comparison of mean VAS scores (0–10) across four categories: comfort, pain (inverted scale), noise perception, and overall satisfaction between GBT and Standard Protocol groups.

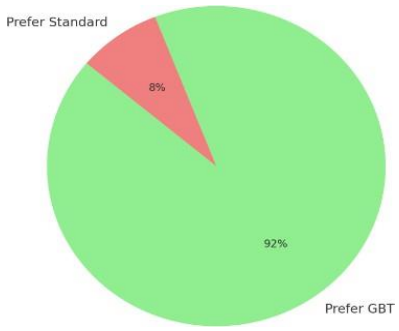


Figure 4. of patient preference between Guided Biofilm Therapy and the traditional standard protocol after undergoing hygiene treatment.

4. CONCLUSION

This observational study confirms that Guided Biofilm Therapy (GBT) provides a superior patient experience and greater clinical efficiency compared to the standard protocol for professional oral hygiene. The statistically and clinically significant differences in pain perception, comfort, satisfaction, and treatment time highlight GBT as a more favorable approach from both the patient's and operator's perspective.

Patients treated with GBT consistently reported lower discomfort and greater tolerance to instrumentation and noise. The visual disclosing phase and use of minimally invasive devices contributed to increased patient engagement and adherence, elements that are often overlooked in traditional hygiene protocols. The high preference rate for GBT suggests that patient-reported outcomes should be more routinely considered in evaluating maintenance strategies.

In addition, the ergonomic benefits observed by the clinical staff, such as reduced hand fatigue and shorter appointment duration, suggest that GBT may contribute to long-term occupational health among oral health professionals.

Clinical Recommendations

- GBT should be considered the standard of care in periodontal maintenance, especially in patients with dentinal sensitivity, restorative work, or implant-supported prostheses.
- Dental hygienists and clinicians should be trained in GBT to ensure protocol fidelity and maximize its ergonomic and biological benefits.
- Future studies should aim to validate these findings in randomized controlled trials with longer follow-up and clinical endpoints such as bleeding on probing, plaque index, and probing depth.

Incorporating PROMs as a routine measure of treatment success is strongly recommended, as they capture dimensions of care quality that go beyond mechanical biofilm removal and directly impact patient loyalty, motivation, and health outcomes.

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 (SRB/SDC/PhD/PERIO-2251/25/025).

Informed Consent

Verbal and written informed consent were obtained from healthy volunteers

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RESEARCH ARTICLE

NUTRITIONAL STATUS AND SUPPLEMENT USE IN PARALYMPIC ATHLETES IN YOGYAKARTA: INSIGHTS FROM A CROSS-SECTIONAL STUDY

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ABSTRACT

Background: Athletes with disabilities face unique physiological and nutritional challenges that can affect performance, recovery, and overall health. However, limited data exist on dietary intake and nutritional status of Paralympic athletes in low- and middle-income countries, including Indonesia. This study aimed to assess nutritional status, dietary intake, and supplement use among Paralympic athletes in Yogyakarta.

Methods: A cross-sectional descriptive study was conducted among 99 athletes participating in 2024 regional training programme under National Paralympic Committee Yogyakarta. Data were collected through anthropometric measurements, vital sign assessments, structured dietary recall (three-day method), and questionnaires on supplement use and health history. Descriptive statistics and cross-tabulations were used to analyse dietary patterns and nutritional status.

Results: Mean daily energy intake was 1,950.6 kcal (SD ±866.3), with wide inter-individual variation. Carbohydrates contributed most to total energy intake. Micronutrient analysis revealed frequent deficiencies in vitamins A, C, and E, while some athletes exceeded recommended sodium and cholesterol levels. Mean BMI was 23.6 kg/m², with 18.9% classified as underweight and 31.6% as overweight or obese. Supplement use was inconsistent, with multivitamins and vitamin C being the most common. Sport-specific trends indicated both under- and overnutrition risks.

Conclusion: Paralympic athletes in Yogyakarta exhibit diverse and often suboptimal nutritional patterns. Nutritional monitoring, personalised dietary planning, and structured supplement guidance are recommended to optimise performance and health outcomes. Further longitudinal studies are warranted to evaluate long-term effects of targeted nutritional interventions in this population.

Keywords: Paralympic athletes, nutritional status, dietary intake, supplement use, disability.

1. INTRODUCTION

Inclusion of persons with disabilities in health promotion and healthcare services is an essential component of Sustainable Development Goals (SDGs), particularly goal three, which targets good health and well-being for all.[1] Sport plays a multifaceted role in lives of individuals with disabilities, serving not only as a means of physical rehabilitation and social integration but also as pathway to high-performance athletic achievement through paralympic competition.[2] Adequate nutrition is foundational element in development and performance of athletes, including those with disabilities, who may face unique physiological and metabolic challenges.[3,4] Paralympic athletes often require tailored nutritional strategies due to differences in muscle mass, energy expenditure, and disability-specific needs. For instance, athletes with spinal cord injuries may have reduced energy expenditure due to lower muscle mass, whereas amputee athletes may have altered energy demands related to gait or prosthesis use.[5] Despite these distinct considerations, research on nutritional status and dietary practices of athletes with disabilities remains limited, especially in low- and middle-income countries.[6,7] Most existing data on sports nutrition come from Western populations and are rarely specific to paralympic athletes. Studies have shown that even among elite paralympic athletes, micronutrient deficiencies particularly in vitamins D, E, iron, calcium, and folate are common and may impact performance and recovery.[8,9] Korean study using 24-hour recall and nutrition quotient scoring revealed that disabled athletes preparing for Tokyo Paralympics had significantly inadequate intake of vitamin C, E, and potassium.[10] In Indonesia, study of disabled athletes in Jakarta reported that 14.4% were undernourished and 26.6% were either overweight or obese, with imbalanced intake of macronutrients, carbohydrate deficits and protein/fat excesses were frequently observed.[11] Given physiological variability among Paralympic athletes and lack of locally contextualised dietary data, routine nutritional assessments are essential. Comprehensive evaluation should include dietary recall, anthropometric measurements, clinical assessment, and biochemical tests where possible, as outlined by current guidelines from American Society for Parenteral and Enteral Nutrition (ASPEN).[12] Moreover, dietary supplement use among this population is not well understood. Although multivitamins, protein powders, and essential fatty acids are commonly used, the prevalence, patterns, and potential benefits of supplementation remain under-investigated in athletes with disabilities.[8,13] This study aims to assess the nutritional status, dietary intake patterns, and supplement use among Paralympic athletes in Yogyakarta, Indonesia. By identifying nutritional imbalances, this study seeks to inform targeted interventions to improve health, performance, and recovery in this underrepresented population.

METHODS

Study Design and Setting

This study employed a descriptive analytical design with a cross-sectional approach. It was conducted in Special Region of Yogyakarta, Indonesia, involving athletes under supervision of Yogyakarta branch of National Paralympic Committee (NPC). Research was carried out as part of regional training programme (Pelatda) for year 2024.

Participants and Sampling

Study population comprised all paralympic athletes registered with NPC Yogyakarta. Participants were selected using purposive sampling based on the following inclusion criteria: (1) athletes officially enrolled in 2024 Pelatda programme, (2) aged ≥15 years, and (3) willing to provide informed consent. Exclusion criteria included acute illness or unwillingness to participate. Total of 99 athletes met eligibility criteria and were enrolled in the study. Sample size was determined based on total population availability of paralympic athletes in the region.

Data Collection

Data were collected through a combination of direct physical measurements and structured questionnaires administered by trained researchers. Data collection procedure included:

1. Anthropometric assessments, including body weight (kg), height (cm), sitting height, mid-upper arm circumference (MUAC), and waist circumference, following WHO standard procedures.[14]
2. Vital sign evaluation, including systolic and diastolic blood pressure, using digital sphygmomanometers with participants at rest.
3. Dietary assessment using three-day food recall method, which involved documenting all foods and beverages consumed during two weekdays and one weekend day. Trained enumerators guided participants in estimating portion sizes using standard food models and measurement aids.
4. Supplement use and health history questionnaire, adapted from previously validated surveys,[15,16] recorded information on supplement intake (type, frequency), injury history, and chronic medical conditions.

Key variables measured in this study included:

1. Nutritional status, assessed using Body Mass Index (BMI) and categorized according to WHO criteria (underweight <18.5, normal 18.5–24.9, overweight ≥25).
2. Macronutrient and micronutrient intake, derived from dietary recall and analyzed using Indonesian Food Composition Tables (DKBM) and Nutrisurvey software.
3. Supplement use, reported as categorical data (yes/no) for each supplement type.
4. Health and injury history, self-reported.

All data were processed and analyzed using SPSS software version 16.0 (IBM Corp, Armonk, NY). Descriptive statistics were employed to summarize demographic variables, nutritional intake (mean, standard deviation, minimum, and maximum), and supplement use frequency. No inferential tests were conducted as the study aimed to describe population-level characteristics rather than establish causal associations. However, cross-tabulations were performed to explore trends between categorical variables, such as BMI categories and supplement use, across different sports disciplines. This study adhered to ethical principles outlined in the Declaration of Helsinki and received ethical clearance from institutional ethics committee of Universitas Negeri Yogyakarta. Report adheres to STROBE checklist.

RESULTS

Participant Characteristics

Total of 99 Paralympic athletes participated in the study. Majority were male (64.6%), and predominant types of disabilities included lower limb physical impairment (63.6%), followed by visual impairment (21.2%). Athletes represented 10 different sports disciplines, with archery (18.2%) and table tennis (15.2%) having the largest proportions. Mean duration of athletic experience was 7.1 years (SD ±5.1), with a wide age range reflecting both junior and senior athletes.

Anthropometric and Clinical Findings

Anthropometric assessments showed mean height of 158.6 cm (SD ±11.1) and mean body weight of 59.8 kg (SD ±14.4). Mean Body Mass Index (BMI) was 23.6 kg/m² (SD ±4.7), ranging from 14.7 to 40.4. Based on WHO classification, 18 athletes (18.9%) were underweight, 47 (49.5%) had normal BMI, 22 (23.2%) were overweight, and 8 (8.4%) were classified as obese.

Vital signs assessment indicated that most athletes had normal systolic and diastolic blood pressure; however, a few individuals exhibited elevated readings, particularly among weightlifters and table tennis players, possibly associated with high sodium intake and BMI levels.

Dietary Intake and Nutritional Status

Analysis of dietary recall data over three days (table 1) revealed a wide variability in energy and nutrient intake. Mean daily energy intake was 1,950.6 kcal (SD ±866.3), with extremes ranging from 431 kcal to 5,104 kcal. Carbohydrate intake contributed majority of daily calories (mean 242.4 g/day), followed by fat (mean 73.1 g/day) and protein (mean 84.0 g/day). Some athletes demonstrated extremely low energy intakes (below 1,000 kcal/day), particularly in archery and boccia, raising concerns about undernutrition and inadequate recovery. Conversely, athletes in weightlifting and judo disciplines often reported excessive energy intake (>3,000 kcal/day), accompanied by high fat and cholesterol consumption. Micronutrient intake showed inconsistent patterns. Mean vitamin A intake was 1,510 µg (SD ±2,989), but some athletes had zero intake. Similar deficiencies were noted for vitamin C (mean 52.7 mg/day, SD ±137.8) and vitamin E (mean 6.9 mg/day, SD ±6.6). High standard deviations indicated substantial inter-individual variation. Sodium intake averaged 611.5 mg/day (SD ±908.7), with some athletes consuming over 5,800 mg/day, more than double WHO recommended upper limit.

Supplement Use

Notable portion of athletes reported irregular or no use of dietary supplements. Among those who did, most commonly consumed supplements were multivitamins, vitamin C, vitamin B1, folic acid, and protein powders. However, adherence and frequency of use varied by discipline and individual preference. Athletes from weightlifting and judo were more likely to use supplements compared to those from boccia or chess.

Sport-Specific Observations

1. Weightlifting athletes had the highest average BMI (29.1 kg/m²) and reported the highest daily energy intake. Several athletes exceeded daily recommendations for sodium, cholesterol, and calories, with limited intake of antioxidant vitamins.
2. Archery athletes, the largest group, exhibited wide variation in energy intake (600–3,400 kcal) and nutritional status, with some showing signs of undernutrition.
3. Table tennis athletes had normal average BMI but presented with abdominal obesity in some cases, supported by high cholesterol and saturated fat intake.
4. Boccia and chess athletes demonstrate lowest average energy intake and multiple micronutrient deficiencies.
5. Judo athletes recorded highest potassium and protein intakes, with elevated sodium and caloric intake contributing to above-normal BMI levels in several cases.

Table 1. Descriptive statistics of key dietary intake variables among 99 Paralympic athletes in Yogyakarta, based on three-day dietary recall data.

	Minimum	Maximum	Mean	Std. Deviation
Energy	431.0	5104.0	1950.648	866.3388
Protein	11.4	217.0	84.013	41.7096
Fat	3.4	222.4	73.096	50.5791
Carbohydrate	27.6	703.8	242.352	120.8865
Dietary fiber	0.2	45.6	13.296	8.6874
Alcohol	0	0	.00	.000
PUFA	0.6	85.8	18.335	16.6133
Cholesterol	0.0	3926.4	508.177	562.2678
Vit. A	0.0	22650.0	1510.098	2989.1439
Carotene	0.0	1.0	0.015	0.1095
Vit. E (eq.)	0.0	35.8	6.912	6.6211
Vit. B1	0.2	2.2	0.696	0.3716
Vit. B2	0.2	5.4	1.342	1.1373
Vit. B6	0.4	5.2	1.312	0.7764
Tot. fol.acid	18.4	1181.8	243.956	188.3133
Vit. C	0.0	1023.8	52.683	137.7715
Sodium	8.0	5855.6	611.504	908.7352
Potassium	248.0	19862.0	2354.573	3441.3977
Calcium	25.2	3725.8	441.758	532.1209
Magnesium	53.8	2335.0	348.283	408.4552
Phosphorus	189.0	4165.2	1068.383	616.3957
Iron	2.4	65.4	12.419	12.1768
Zinc	2.0	25.0	8.521	4.3660

4.DISCUSSION

Findings revealed considerable variability in energy and micronutrient intake across different sport disciplines, suggesting that current dietary practices may be suboptimal for supporting peak athletic performance and recovery. This aligns with prior research noting that athletes with disabilities often face unique nutritional challenges that are not adequately addressed in standard sports nutrition guidelines.[4,6] Mean daily energy intake of 1,950 kcal in this study appears insufficient for high-performance athletes, particularly given their elevated energy expenditure during intensive training. Notably, some athletes consumed less than 1,000 kcal/day, which is concerning when viewed in the context of minimum energy availability required to maintain physiological and cognitive function in athletes.[9] Chronic energy deficits have been linked to reduced muscle mass, impaired immune function, hormonal imbalances, and increased risk of injury.[13,17] In contrast, other athletes, particularly in weightlifting and judo, consumed more than 3,000 kcal/day, suggesting a mismatch between energy intake and expenditure, potentially increasing risk of adiposity and metabolic strain.[5] Dominance of carbohydrates as a primary energy source is consistent with national dietary patterns in Indonesia.[18] However, wide standard deviations in macronutrient intake indicate inconsistent meal planning and possible lack of nutritional supervision.

Protein intake varied widely (11–217 g/day), which may reflect differences in sport demands or access to protein-rich foods and supplements. Although average intake may appear adequate, quality and timing of protein consumption are crucial for muscle repair and training adaptation.[19]

Micronutrient intake presented further concerns. Deficiencies in vitamin C, E, and A were observed in several athletes, despite these nutrients playing vital roles in immune function, antioxidant defence, and tissue repair.[20] These findings echo earlier studies of Paralympic athletes, such as Madden et al., which reported frequent shortfalls in micronutrients among both male and female athletes.[5] Notably, 2021 Korean Paralympic nutrition study reported similar deficiencies in vitamin E and potassium intake among athletes with disabilities, supporting generalisability of present findings to other Asian contexts.[10] Excessive sodium intake was a notable finding, particularly among weightlifters and judo athletes, where daily intake surpassed 5,000 mg in some cases. World Health Organization recommends an upper limit of 2,300 mg/day for adults, with excess intake linked to elevated blood pressure and increased cardiovascular risk.[21] This may explain elevated systolic and diastolic blood pressure observed in several athletes.

Salt-rich processed foods and seasoning-heavy diets common in Indonesian culture may contribute to this pattern, warranting nutritional education and dietary counselling. Use of dietary supplements was inconsistent. While some athletes used multivitamins and protein powders, overall supplementation adherence was low, and few athletes reported regular intake of micronutrient-based supplements. This may reflect limited access, financial constraints, or a lack of nutrition education. Previous studies indicate that appropriate use of supplements can support performance, particularly in athletes with restricted dietary diversity or increased physiological demands.[22] However, unregulated supplement use without professional guidance carries potential risks, including toxicity and contamination.[23] Sport-specific observations provided useful insights. For instance, archery athletes exhibited low BMI and energy intake despite the need for mental endurance and postural stability, which require consistent energy availability.[24] In contrast, weightlifters exhibited high BMI and caloric intake but low antioxidant consumption, highlighting risk of overnutrition coexisting with micronutrient deficiencies. Table tennis athletes displayed indications of abdominal obesity despite normal average BMI, emphasizing the importance of waist circumference as a complementary indicator of cardiometabolic risk.[25]

Strengths and Limitations

Key strength of this study lies in its use of primary data collection across a large and diverse sample of athletes with disabilities, including a broad representation of sport disciplines. Additionally, combination of anthropometric data, dietary recall, and supplement use provides a multidimensional view of nutritional status. However, several limitations should be noted. First, three-day dietary recall method, while practical, is prone to recall bias and may not accurately reflect habitual intake.[26] Second, the cross-sectional nature of the study prevents causal inference regarding the relationship between dietary intake and performance or health outcomes. Third, unequal distribution across sports disciplines may limit the generalisability of sport-specific findings.

Implications

These findings underscore urgent need for targeted nutritional interventions and monitoring in Paralympic training programmes. Institutional support for individualized meal planning, regular assessment of micronutrient status, and professionaldietary counselling is recommended. Given physiological variability associated with different disabilities, future research should adopt longitudinal designs and incorporate biochemical assessments to evaluate impact of tailored nutrition on athletic performance and recovery.

CONCLUSION

This study highlights substantial variability in nutritional status, dietary intake, and supplement use among Paralympic athletes in Yogyakarta. While some athletes demonstrated adequate energy and macronutrient intake, others exhibited patterns of undernutrition or overnutrition, often accompanied by micronutrient imbalances. Inconsistent use of dietary supplements and presence of sport-specific nutritional risks underscore the need for individualized nutritional assessment and guidance. Tailored dietary interventions, combined with structured nutrition education, may enhance athletic performance, reduce injury risk, and support long-term health in this underrepresented athletic population. Future research should adopt longitudinal and biomarker-based approaches to further investigate the impact of dietary strategies on functional and competitive outcomes in athletes with disabilities.

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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EVALUATION OF POSTOPERATIVE PAIN AND ANXIETY IN PEDIATRIC PATIENTS UNDERGOING ORAL SURGERY WITH LOCAL ANESTHESIA ALONE VS. CONSCIOUS SEDATION

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ABSTRACT

This study evaluates the postoperative pain and anxiety levels in pediatric patients undergoing oral surgery under two anesthetic modalities: local anesthesia alone and local anesthesia combined with conscious sedation. Pediatric dental procedures often pose challenges due to the psychological and physiological responses of children, especially concerning pain and anxiety management. The study is a prospective, randomized, controlled clinical trial that investigates how these two approaches affect postoperative outcomes. A total of 70 children, aged 4 to 12 years, were divided into two groups: one receiving only local anesthesia and the other receiving local anesthesia combined with conscious sedation (using agents like midazolam or nitrous oxide). Postoperative pain and anxiety were assessed using the Wong-Baker FACES Pain Rating Scale and the Modified Yale Preoperative Anxiety Scale, respectively, at two key time points: immediately post-surgery and 24 hours after the procedure. The results indicated that the conscious sedation group reported significantly lower pain and anxiety scores immediately post-surgery and 24 hours later, compared to the local anesthesia-only group. Furthermore, the sedation group required fewer postoperative analgesics and showed higher caregiver satisfaction. These findings suggest that conscious sedation, when appropriately used, can significantly enhance patient comfort, alleviate anxiety, and improve overall postoperative outcomes. This study emphasizes the importance of addressing both physical and psychological aspects of care in pediatric oral surgery.

Keywords: Anxiety, Conscious sedation, Local anesthesia, Pediatric dentistry, Postoperative care

1.INTRODUCTION

Pediatric dental surgery poses unique challenges due to the physiological and psychological characteristics of young patients [1]. Children often experience heightened levels of fear and anxiety before and during dental procedures, which can significantly influence their pain perception, cooperation,

and overall treatment outcomes [2]. Among these procedures, oral surgery—including tooth extractions, frenectomies, and soft tissue surgeries can be particularly distressing. Consequently, managing both pain and anxiety in pediatric patients is of paramount importance for clinicians aiming to deliver effective and compassionate care [3].

Local anesthesia has long been the standard method for providing pain control during minor oral surgical procedures. It blocks nerve conduction at the site of intervention, ensuring the procedure itself is physically painless [4]. However, local anesthesia does not address the psychological component of the experience namely, the child's fear and anxiety [5]. The sight of needles, unfamiliar sounds, and a clinical environment can provoke significant distress in children, leading to increased pain perception, uncooperative behavior, and even long-term dental phobias [6].

To mitigate these issues, conscious sedation has been increasingly employed in pediatric dentistry. Conscious sedation involves the use of pharmacological agents to depress the central nervous system to a level where the patient is still able to respond to verbal commands but is significantly more relaxed and less aware of the procedure [7]. Common agents include midazolam, nitrous oxide, and ketamine, often used alone or in combination. Conscious sedation not only reduces anxiety but may also have an indirect effect on pain perception by altering the patient's emotional response and threshold for discomfort [8].

Despite its growing popularity, the use of conscious sedation in pediatric dental procedures remains a topic of debate. Concerns around safety, cost, and the need for specialized training and equipment have led some practitioners to rely solely on local anesthesia [9]. However, there is growing evidence suggesting that conscious sedation, when used appropriately, can significantly enhance patient comfort, cooperation, and satisfaction factors that may influence both immediate and long-term clinical outcomes [10].

Postoperative pain and anxiety are important considerations that can affect recovery, caregiver satisfaction, and a child's future attitudes toward dental care. Pain, if poorly managed, can result in disrupted sleep, difficulty eating, and behavioral issues [11]. Anxiety following a negative surgical experience may lead to dental avoidance behaviors, complicating future treatment needs. While numerous studies have examined intraoperative pain and behavioral responses, fewer have focused on the postoperative period, especially comparing outcomes between children treated with local anesthesia alone versus those receiving conscious sedation [12].

The present study aims to evaluate the postoperative pain and anxiety levels in pediatric patients undergoing oral surgery under two different anesthetic modalities: local anesthesia alone and local anesthesia combined with conscious sedation [13]. By comparing these two approaches, this study seeks to provide evidence-based insights into the effectiveness of sedation in improving postoperative outcomes. Specifically, it will investigate whether conscious sedation contributes to lower reported pain levels, reduced anxiety, and improved overall patient and caregiver satisfaction in the days following surgery.

This research is timely and significant given the increasing demand for pediatric dental services and the need for patient-centered care strategies . Understanding the impact of different anesthetic approaches on postoperative well-being can help dental practitioners make informed decisions about pain and anxiety management, ultimately fostering a more positive healthcare experience for young patients. Moreover, the findings could inform clinical guidelines and training programs, promoting safer and more effective use of conscious sedation in pediatric oral surgery.

Methodology

This prospective, randomized, controlled clinical trial aimed to evaluate and compare postoperative pain and anxiety in pediatric patients undergoing oral surgery with either local anesthesia alone or local anesthesia combined with conscious sedation. The study adhered to ethical standards in accordance with the Declaration of Helsinki and was approved by the institutional review board (IRB) prior to commencement. Written informed consent was obtained from the legal guardians of all pediatric participants, with verbal assent from the patients when appropriate.

Study Design and Setting

The study was conducted in a pediatric dental clinic affiliated with a university dental school. The population consisted of children aged 4-12 years who were scheduled to undergo routine oral surgical procedures, such as tooth extractions, frenectomies, or soft tissue biopsies. These procedures were classified as minor oral surgery requiring either local anesthesia or conscious sedation for adequate pain management.

Inclusion and Exclusion Criteria

Inclusion Criteria:

- Children aged 4-12 years
- Scheduled for a minor oral surgical procedure (e.g., tooth extraction, frenectomy)
- ASA (American Society of Anesthesiologists) physical status I or II

- Guardians who provided written informed consent
- Ability of the child to understand and cooperate with pain and anxiety assessments

Exclusion Criteria:

- History of adverse reactions to sedative agents or local anesthesia
- Severe dental anxiety (as determined by the Frankl Behavioral Rating Scale)
- Severe medical conditions that contraindicate sedation (e.g., respiratory or cardiovascular disorders)
- Children who required general anesthesia for their procedure

Sample Size Calculation

The sample size was calculated based on the primary outcome of postoperative pain scores, assuming a medium effect size (Cohen's d = 0.5) between the two groups and a power of 80% with an alpha level of 0.05. Using these parameters, a total of 60 participants (30 in each group) were determined to be required to detect a statistically significant difference in pain and anxiety outcomes between the two anesthetic approaches. The sample size was adjusted to account for potential dropouts, with a target of recruiting 70 participants in total.

Randomization and Group Allocation

Upon arrival at the clinic, eligible patients were randomly assigned to one of two intervention groups using a computer-generated randomization list. The two groups were:

Group 1 (Local Anesthesia Only): Patients received a standard local anesthetic (e.g., lidocaine 2% with epinephrine) at the surgical site without any sedative agents.

Group 2 (Local Anesthesia with Conscious Sedation): Patients received a local anesthetic (e.g., lidocaine 2% with epinephrine) in combination with conscious sedation using a pharmacological agent, such as oral midazolam (0.5 mg/kg) or nitrous oxide.

The allocation sequence was concealed from both the clinician administering the anesthetic and the participants to reduce bias.

Procedure Protocol

For all patients, the procedures were performed by a single experienced pediatric oral surgeon. For patients in Group 1, the surgeon administered the local anesthetic, allowing sufficient time for the anesthesia to take effect before commencing surgery. For patients in Group 2, the sedation was administered orally or via nitrous oxide inhalation 30 minutes before the surgical procedure to ensure adequate sedation.

Standard monitoring, including pulse oximetry, heart rate, and blood pressure, was performed on patients in Group 2 who received conscious sedation. In both groups, the surgery proceeded once the patient was confirmed to be adequately anesthetized, with careful attention to maintaining patient comfort throughout the procedure.

Postoperative Pain and Anxiety Assessment

Pain and anxiety were evaluated at two key time points: immediately post-surgery (within 30 minutes) and 24 hours after the procedure.

Pain Assessment: The Wong-Baker FACES Pain Rating Scale was used immediately after surgery and 24 hours postoperatively to assess pain intensity. This scale is validated for use in pediatric populations and provides an easily understandable visual tool for children to rate their pain. Parents or guardians also completed a pain assessment for the child using the same scale.

Anxiety Assessment: Anxiety levels were measured using the Modified Yale Preoperative Anxiety Scale (mYPAS), which has been widely used in pediatric dental research to quantify anxiety levels in children before and after treatment. The scale assessed various indicators of anxiety, including behavioral and physiological responses. Parents also completed an anxiety assessment based on their child's behavior before and after the procedure.

Data Collection and Statistical Analysis

Data were collected by trained research assistants who were blinded to the treatment group allocation. The primary outcome measures were the pain scores and anxiety scores at the two postoperative time points. Secondary outcomes included the need for additional pain relief (e.g., postoperative analgesic use) and patient or parent satisfaction with the procedure.

Statistical analysis was performed using SPSS version 27. Descriptive statistics were used to summarize patient characteristics, pain, and anxiety scores. Comparisons between the two groups for pain and anxiety outcomes were conducted using independent

t-tests or Mann-Whitney U tests for non-parametric data. A p-value of < 0.05 was considered statistically significant.

Ethical Considerations

The study ensured the safety and well-being of all pediatric participants. All procedures, including the use of sedation agents, were performed in compliance with established safety guidelines. Any adverse events were monitored and managed in accordance with standard protocols. Participants were closely observed during and after the procedure to ensure their safety and comfort.

RESULTS

A total of 70 pediatric patients (35 in each group) were included in the study. Of the 70 patients enrolled, five were excluded due to incomplete data or withdrawal from the study, leaving 65 patients for analysis (32 in the local anesthesia-only group and 33 in the regional anesthesia with conscious sedation group). The baseline demographic characteristics of the two groups were similar, with no significant differences in age, sex, or ASA physical status (Table 1).

Pain Scores

Pain levels were measured immediately after surgery and 24 hours postoperatively using the Wong-Baker FACES Pain Rating Scale. The average pain score immediately post-surgery for the local anesthesia group was significantly higher compared to the conscious sedation group. Specifically, the local anesthesia-only group had a mean pain score of 6.4 (± 1.2), whereas the conscious sedation group reported a mean score of 3.2 (± 1.1) ($p < 0.001$).

At 24 hours post-surgery, pain scores in both groups decreased, but the difference between groups remained significant. The local anesthesia group reported a mean score of 3.9 (± 1.0), while the conscious sedation group had a mean score of 2.3 (± 0.9) ($p = 0.004$).

Table 1. Baseline Demographics of Participants

Demographic Variable	Local Anesthesia Only (n = 32)	Local Anesthesia + Sedation (n = 33)	p-value
Age (mean ± SD)	8.2 ± 2.1 years	8.0 ± 1.9 years	0.68
Gender (M/F)	16/16	17/16	0.88
ASA Physical Status I/II	32/0	32/1	0.39

Table 2. Pain Scores in Both Groups

Time Point	Local Anesthesia Only (n = 32)	Local Anesthesia + Sedation (n = 33)	p-value
Immediately Post-Surgery	6.4 ± 1.2	3.2 ± 1.1	< 0.001
24 Hours Post-Surgery	3.9 ± 1.0	2.3 ± 0.9	0.004

Anxiety Scores

Anxiety levels, measured immediately post-surgery and 24 hours post-surgery using the Modified Yale Preoperative Anxiety Scale (mYPAS), showed a similar pattern. Immediately after surgery, the local anesthesia-only group had a significantly higher anxiety score (mean 37.6 ± 8.5) compared to the conscious sedation group (mean 21.4 ± 7.9) ($p < 0.001$). At 24 hours, the local anesthesia group's anxiety score decreased to 26.3 ± 7.2, while the conscious sedation group's anxiety score was 18.1 ± 6.3, maintaining a significant difference ($p = 0.001$).

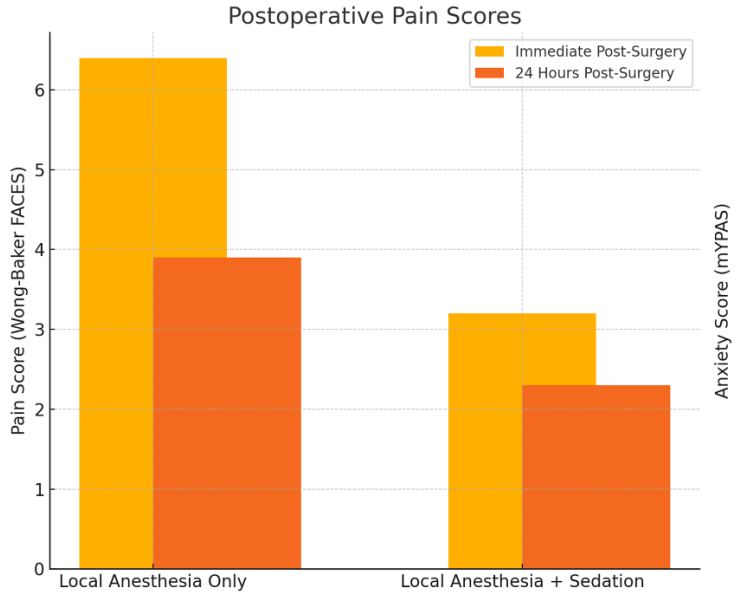
Table 3. Anxiety Scores in Both Groups

Time Point	Local Anesthesia Only (n = 32)	Local Anesthesia + Sedation (n = 33)	p-value
Immediately Post-Surgery	37.6 ± 8.5	21.4 ± 7.9	< 0.001
24 Hours Post-Surgery	26.3 ± 7.2	18.1 ± 6.3	0.001

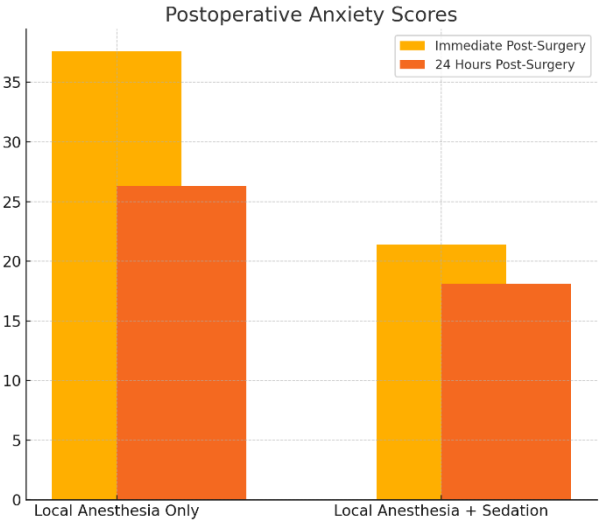
Secondary Outcomes

Postoperative Analgesic Use: The need for postoperative analgesics was significantly higher in the local anesthesia-only group, with 70% of patients requiring additional pain medication within the first 24 hours. In contrast, only 36% of patients in the conscious sedation group required additional analgesics (p = 0.002).

Patient and Caregiver Satisfaction: Satisfaction scores were collected using a 5-point Likert scale (1 = very dissatisfied, 5 = very satisfied). The conscious sedation group had higher satisfaction scores, with a mean score of 4.5 ± 0.6 compared to 3.3 ± 1.0 for the local anesthesia-only group (p < 0.001).



Graph 1. Postoperative Pain Scores in Both Groups



Graph 2. Anxiety Scores in Both Groups

4. DISCUSSION

The finding that conscious sedation significantly reduced pain scores immediately post-surgery and 24 hours post-surgery aligns with similar studies in the pediatric dental literature. For example, a study by **Khole M et al. (2025)** [13] found that children who received nitrous oxide in combination with local anesthesia reported significantly lower pain scores after tooth extraction compared to those who received only local anesthesia. In that study, the analgesic effect of nitrous oxide was attributed to its anxiolytic properties, which reduced the overall perception of pain.

Our study also found that the conscious sedation group required fewer additional analgesics in the 24 hours following the procedure. This is in line with research by **Bean T et al. (2025)** [6], which showed that children who underwent dental procedures with conscious sedation, particularly using agents like midazolam, reported a greater sense of comfort and required less postoperative analgesia. The reduced need for additional pain medications in the sedation group could be attributed to the enhanced relaxation and analgesic effects of sedative agents, which may have altered pain thresholds.

In contrast, the local anesthesia-only group in the present study reported higher pain scores and required more analgesic intervention, supporting the notion that local anesthesia, while effective during the procedure itself, does not fully address postoperative discomfort in pediatric patients. **Weinstein EJ et al**. (2018) [14] similarly noted that while local anesthesia alone can effectively manage intraoperative pain, it often falls short in controlling postoperative pain and anxiety in children.

The reduction in anxiety observed in the conscious sedation group is consistent with findings from **Ashley PF et al. (2018)** [15], who demonstrated that children receiving sedation, particularly oral midazolam, reported lower anxiety levels during and after dental procedures. The anxiolytic effects of conscious sedation, including midazolam and nitrous oxide, likely contributed to this reduction. The present study found that the conscious sedation group had significantly lower anxiety scores both immediately after surgery and 24 hours post-surgery compared to the local anesthesia-only group. This is in line with the results from **Wilson TD et al. (2014)** [16], who concluded that sedation with nitrous oxide in combination with local anesthesia led to a more relaxed and less fearful experience for pediatric patients, which persisted into the postoperative period.On the other hand, children who received only local anesthesia in the present study exhibited higher anxiety scores, which likely reflect the discomfort and stress associated with both the surgical procedure and the lack of psychological support. **Eijlers R et al. (2021)** [17] noted that children undergoing invasive dental procedures without sedation were more likely to experience heightened anxiety, which can negatively impact their recovery and future dental experiences.

Limitations and Future Research

This study has several limitations. The sample size, while calculated to provide adequate power, was relatively small, and the findings may not be generalizable to all pediatric populations. Additionally, the study focused on only a limited set of postoperative outcomes (pain, anxiety, and satisfaction), and other factors such as behavioral changes, longer-term anxiety, and potential side effects of sedation agents were not assessed. Future research should involve larger, multicenter trials with a broader range of outcome measures, including longer-term follow-ups to assess the persistence of anxiety and pain reduction. Additionally, exploring the use of different sedation agents and comparing their effects on various pain and anxiety outcomes would be valuable. While conscious sedation requires specialized training and equipment, its benefits make it a valuable option in pediatric dental care. Future research should explore long-term effects and the safety of various sedation agents. Additionally, emerging technologies like metaverse AI, AR, and VR hold promise in creating virtual environments for anxiety reduction, offering immersive distraction techniques during procedures and enhancing preoperative education [18]. Integrating these technologies could revolutionize the pediatric dental experience, further reducing reliance on pharmacological sedation and improving patient outcomes [19,20].

Conclusion

In this study, conscious sedation significantly reduced postoperative pain and anxiety compared to local anesthesia alone in pediatric patients undergoing oral surgery. The conscious sedation group reported lower pain and anxiety scores, along with higher overall satisfaction, indicating its effectiveness in improving the postoperative experience. These findings align with previous studies supporting the use of sedation in pediatric dental procedures. Conscious sedation not only addresses pain but also alleviates anxiety, promoting a more comfortable recovery. While conscious sedation requires specialized training and equipment, its benefits make it a valuable option in pediatric dental care. Future research should explore long-term effects and the safety of various sedation agents. Overall, this study underscores the importance of considering both physical and psychological factors in pediatric oral surgery, ultimately improving patient outcomes and satisfaction.

DECLARATIONS

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Consent for publication

Informed consent was obtained from every participant for documentation and examination.

Competing interests

The authors declare no competing interests.

Ethical approval

Ethical approval was granted by the Institutional Human Ethical Committee

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ARTIFICIAL INTELLIGENCE AS AN EMERGING PARADIGM IN ORAL AND MAXILLOFACIAL SURGERY: A QUESTIONNAIRE-BASED STUDY IN KERALA.

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ABSTRACT

Objective: The objective of this study was to evaluate the attitude and perceptions of undergraduate and postgraduate dental students, and practicing clinicians towards the adoption of Artificial Intelligence in the Oral and Maxillofacial surgery curriculum.

Materials and Methods: To conduct a study on this, the online structured questionnaires administered through Google Forms were used in the state of Kerala in India during a period of 3 months. The questions of interest were about participants' knowledge of artificial intelligence, the perceived benefits and drawbacks of its use in academic or clinical contexts.

Results: A total of 300 respondents (100 undergraduates, 100 postgraduates, and 100 clinicians) completed the survey. All groups appeared to have a generally positive outlook on the integration of artificial intelligence. While the clinical benefits were highlighted, concerns were expressed by postgraduate students regarding the complexity and potential inaccuracies of artificial intelligence-generated results. Artificial Intelligence was regarded by practitioners as a tool to improve treatment quality, but it was also perceived to face issues such as a shortage of awareness, high training requirements, and public acceptance.

Conclusion: Finally, the study shows that artificial intelligence is perceived broadly as beneficial as an adjunct in the education and practice of dentistry. Insights generated above can be used as a valuable baseline to devise educational and clinical interventions aiming at humanizing the progression of artificial intelligence in oral healthcare delivery systems.

Keywords**:** AI, Oral surgery, Dental surgery, Questionnaires.

1. INTRODUCTION

Healthcare is a promising domain of Artificial Intelligence. Artificial Intelligence technologies have also been applied in recent years in dentistry to diagnose, plan the treatment, use robotic-assisted surgeries, and manage patient data. Artificial Intelligence tools can simulate human intelligence to analyze large datasets and identify patterns. For example, the integration of the Computer-Aided Design and Computer-Aided Manufacturing (CAD/CAM) systems, RFID-equipped instruments, sensor-based gloves, and robot patients is currently helping to reshape the traditional teaching and practice of Oral and maxillofacial surgery.

The COVID-19 pandemic accelerated the rate of digital transformation in dental education as well as in practice. Since you could not connect through physical means, dental institutions started utilizing their online platforms, simulations, and algorithms-enabled tools to continue on with learning and clinical training [1].

The main focus is often technical capabilities of artificial intelligence, but rarely psychological, educational, and practical readiness of its users [2]. The present study aims to fill this gap by investigating the perceptions and attitudes of undergraduate and postgraduate dental students, as well as practicing clinicians in Kerala, towards the use of Artificial Intelligence in the Oral and Maxillofacial Surgery curriculum and its application in practice. For developing appropriate strategies for facilitating the smooth integration of artificial intelligence, it is important to understand how future and current dental

professionals envision artificial intelligence.

MATERIALS AND METHODS

Study Design

The study is an original research, which adopted a questionnaire with an exploratory approach. The research methodology was devised to probe the nuance in the opinions of three participant categories, namely, undergraduate students, postgraduate students, and regular dental clinicians in Kerala state of India. The questionnaires developed and distributed through Google Forms were used to collect data [1]. Because the study was qualitative, open-ended responses and selected choices could be subjected to thematic interpretation [2].

Study Setting and Participants

The study was done over three months in Kerala, a state in India. This was selected because there is a multiplicity of dental institutions and practitioners in the region. Undergraduate and postgraduate dental students in recognized dental institutions and licensed dental practitioners practicing in the clinical environment were involved as participants. People who could provide relevant and informed perspectives on the subject matter were purposely sampled. In order to be included, participants had to be able to access digital platforms for online completion of the online survey and to have basic knowledge of digital tools [3].

Data Collection

Three different Google Form questionnaires were developed. Formulation of ten multiple-choice questions for each group with regards to participants' awareness, experiences and opinion on the application of artificial intelligence in oral and maxillofacial surgery was involved in the development of survey instruments [4]. In order to get a big and inclusive reach, the distribution of the Google Forms was carried out through institutional mailing lists, faculty referrals, social media platforms and professional dental forums. A brief overview of the study's purpose was given, and they were requested to provide informed consent [5]. The structure of Google Forms ensured anonymity and didn't have anything to do with IP tracking or any contact information. Assured the data would be stored securely and would be used solely for research purposes [6].

Data Analysis

Data will be analyzed using the statistical package SPSS 26.0 (SPSS Inc., Chicago, IL) and level of significance will be set at $p<0.05$. Descriptive statistics will be performed to assess the mean and standard deviation of the respective groups. Normality of the data will be assessed using Shapiro Wilkinson test. Inferential statistics to find out the difference between the groups will be done using Chi Square test.

Independent T test/ Mann Whitney U test will be used for comparison of 2 groups. One way Anova/ Kruskal Wallis test will be used for comparison of > 2 groups. Descriptive thematic analysis was conducted on the data obtained from the Google Form responses. The responses for undergraduates, postgraduates and practitioners were grouped in terms of participant cohorts and were analyzed to establish common themes and trends [7]. To get deeper qualitative insights, open-ended optional feedback and common remarks were used. Initially, the collected data was exported into a spreadsheet (Microsoft Excel) software for first sorting and organizing inside of. Responses were categorized into thematic clusters of 'perceived benefits,' 'concerns about accuracy,' 'training needs,' 'curriculum relevance,' and 'clinical integration,' and pre-coding was done. Counselling frequencies of specific response patterns were also calculated to see which perceptions were most frequently supported across each participant group [8].

Interview Sample Question

For example, a sample question asked of participants was 'Do you think Artificial Intelligence should be taught in the curriculum of Oral and Maxillofacial Surgery to undergraduate and postgraduate dental students?'. The next question directed to clinicians was, "Would you use AI-based diagnostic tools in clinical practice?"

RESULTS

Overview of Findings

The structured Google Forms generated data that revealed the complete picture regarding artificial intelligence perception and attitudes among undergraduate dental students, postgraduate dental students and practicing dental clinicians. The final sample comprised N = 300 respondents: 100 undergraduates, 100 postgraduates, and 100 practicing clinicians. Data showed that everyone among the examined groups expressed positive beliefs regarding integrating artificial intelligence. Undergraduate students saw artificial intelligence primarily as a modern educational instrument. Postgraduate students combined optimism with their belief in artificial intelligence's actual medical value. Artificial Intelligence reliability and possible system boundaries in critical medical scenarios caused them to raise their concerns. The current usage of artificial intelligence by practicing clinicians includes implementing it as an operational tool to enhance treatment efficiency while maintaining treatment quality [4-7]. The way students and dental practitioners feel about artificial intelligence changed depending on their dedication to the field and practice experience [9]. Clinical

professionals revealed significant enthusiasm toward artificial intelligence diagnostics.

All three populations sharing perceptions about artificial intelligence utility matched their evaluations of artificial intelligence complexity, together with requirements for training and ethical awareness within the field [10]. The findings demonstrate mutual agreement about artificial intelligence possibilities for oral and maxillofacial surgery practice, together with fundamental obstacles that need solutions for seamless implementation of artificial intelligence within clinical oral and maxillofacial surgery operations.

Detailed Thematic Findings

Undergraduate Students

A high degree of excitement was noted among undergraduate students regarding artificial intelligence. Undergraduate students primarily supported the idea that automation could simplify their education through interactive learning methods. The students reported that they need to begin their artificial intelligence coursework early because it would help minimize rote learning while developing analytical abilities.

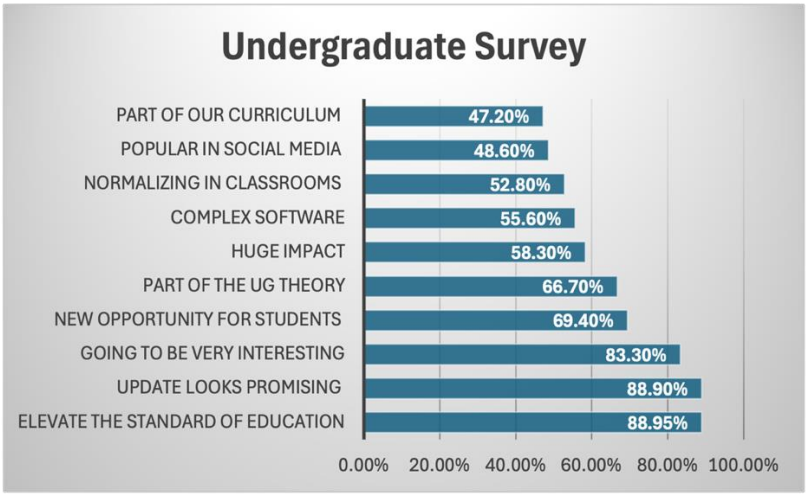


Figure 1. Demonstrates the result of the Undergraduate survey, in which a maximum of 88.95% have an opinion that artificial intelligence will elevate the standard of education. Students believed introducing artificial intelligence would prepare them better to perform in the digitally transformed clinical environment of the future [11]. Artificial intelligence functions as a traditional classroom enhancement system. Their responses demonstrated enthusiasm, but they simultaneously acknowledged the necessity of appropriate training methods.

Postgraduate Students

The postgraduate student population demonstrated a fairly advanced clinical approach to their understanding of artificial intelligence. Students generally focused their answers on how artificial intelligence helps surgeons check and plan operations, as well as deliver personalized medical treatments. The participants in Postgraduate studies stressed that artificial intelligence systems would help manage complicated surgery cases through prediction systems that also reduce errors made by physicians [13].

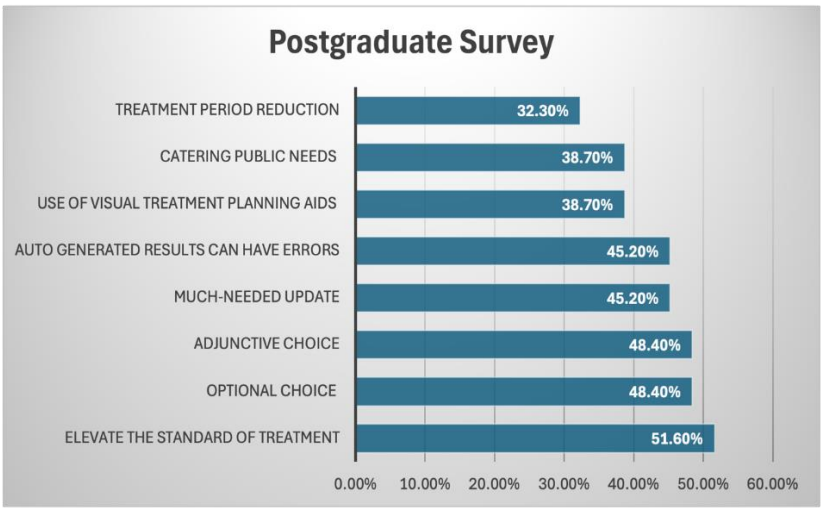


Figure 2. Survey participants from the postgraduate level showed an even temperament when discussing artificial intelligence.

Postgraduate students expressed multiple concerns related to machine dependency and contextual comprehension scarcity [10-13]. A survey participant declared that artificial intelligence technology performs well by helping medical staff, but professionals must maintain authority in situations that need subjective evaluation of patients.
The postgraduate students showed special attention to technical flaws and biased data processing within artificial intelligence algorithms. The participants expressed their concern about implementing artificial intelligence tools unless artificial intelligence tools first receive continuous validation and regulatory oversight [14]. The research team suggested formal artificial intelligence training requirements and education for postgraduate students.

Practicing Clinicians

The dental clinicians who were already practicing the profession viewed artificial intelligence with a practical understanding. Medical staff considered artificial intelligence-assisted radiographic interpretation, together with automated documentation and digital patient record analysis, as the most beneficial uses of artificial intelligence [15]. A practitioner stated that "artificial intelligence enables medical staff to decrease their diagnostic work while enhancing diagnostic precision specific to high-volume treatment facilities."

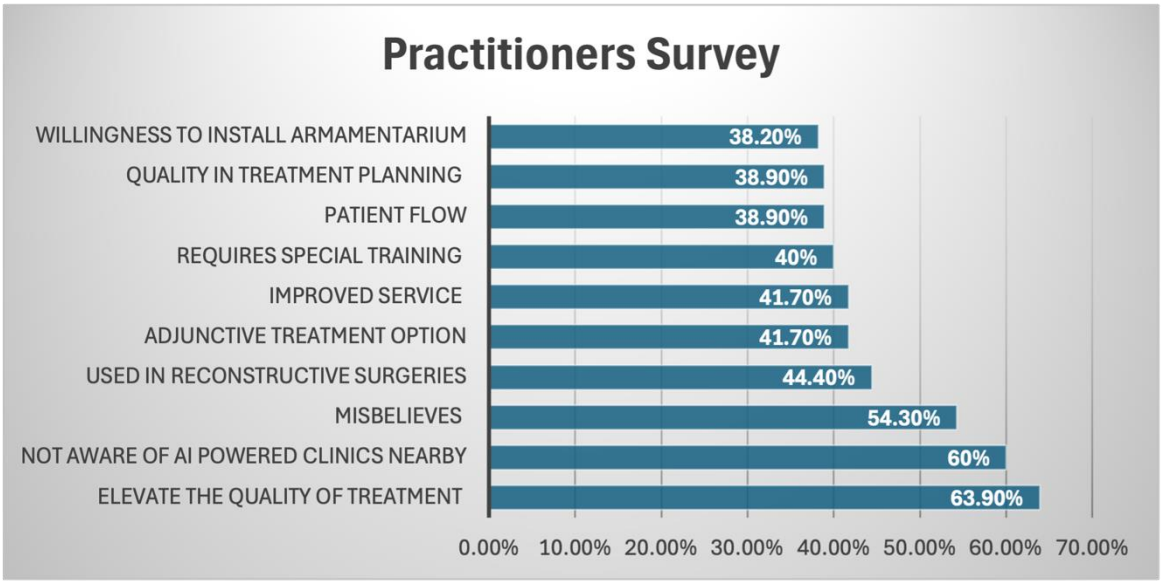


Figure 3. Highlights the ignorance of the general population being unaware of such updates, along with the dilemma existing between the misbeliefs, nearly accounting for 54.30% and specialized training necessities.

The main challenges mainly arose from insufficient formal training for the staff and inadequate technical support systems and patient awareness levels [16]. A study participant stated that patients often do not understand or distrust medical instrumentation. Education and transparency are essential." Practitioners and patients need awareness to create trust in artificial intelligence.

Common Themes

The research revealed common dominant themes. All participants demonstrated a widespread recognition of artificial intelligence utility. The positivity about artificial intelligence implementation met resistance because participants raised questions about tool complexity, with worries about errors and insufficient user education. Multiple participants identified the requirement for structured educational programs as a vital issue [17]. Minimum educational support systems become vital because state-of-the-art tools do not automatically produce desirable results. Postgraduate students and clinicians together highlighted ethical issues.

3. DISCUSSION

Students at both undergraduate and postgraduate levels in dentistry, together with practicing clinicians, show positive attitudes toward implementing Artificial Intelligence within Oral and Maxillofacial Surgery applications. Research findings show that acceptance toward artificial intelligence remains positive among undergraduate and postgraduate students and practitioners, yet understanding differs. The participants' development level and their experience in clinical work, and their current professional demands determine these distinctions [18].

Alignment with Existing Literature

This qualitative research outcome is consistent with academic analysts. Artificial intelligence has become increasingly important for diagnostic imaging, as well as treatment planning and robotic-assisted surgery and

patient management according to research conducted by Muller et al.[10]. The dental field has widely accepted artificial intelligence technology because it improves decision accuracy and enhances minimally invasive procedures, as well as reduces administrative workload [19]. Artificial intelligence diagnostic systems used in dentistry demonstrated similar abilities with radiographic images as trained clinicians, according to Muller et al.[10]. Research literature reveals that Undergraduate and postgraduate students' enthusiasm in this study corresponds with a worldwide pattern where professionals become proficient in digital technologies.

Differing Perspectives Between Undergraduate and Postgraduate Students

The current research shows that undergraduate students welcome Artificial Intelligence as a fresh curriculum element. Their positive attitudes emerged because they believed artificial intelligence systems could simplify difficult anatomical ideas and deliver individualized lesson formats [20].

Postgraduate students demonstrated a blend of positive and reserved outlook. Student experience with real patients influenced postgraduate students to recognize that Artificial Intelligence tools have their practical boundaries and confirmed clinical usefulness. Postgraduate students raised doubts about Artificial Intelligence-based output reliability. Postgraduate students face the unique situation where academic understanding meets real-life clinical demands, which explains their different answers. Multiple participants demonstrated an increased need for evidence and validation from healthcare professionals who work with patients [21].

Practitioners' Hesitation and Real-World Concerns

Medical practitioners demonstrated more extensive caution in their responses. Healthcare personnel acknowledged Artificial Intelligence's positive impacts. The healthcare professionals limited their approval processes through analysis of existing work process constraints [22]. Hospital practitioners brought up three major issues, which included inadequate training for Artificial intelligence systems, difficulties integrating Artificial intelligence routines into existing workflows and patients' doubts about Artificial intelligence systems.

Barriers to Artificial Intelligence Integration

All participant groups highlighted the absence of organized training related to Artificial Intelligence as their primary theme. Practitioners without digital health education during their time at school expressed strong concerns about training in artificial intelligence tools [23]. Healthcare providers indicated that their lack of suitable infrastructure presented a barrier. Practice settings that lack high-end hardware and adequate software integration, along with reliable technical support, cannot implement advanced artificial intelligence tools.

Members of the study voiced their concern because patients generally show little understanding of artificial intelligence systems. Developing trust in patients represents an essential consideration because patient trust affects their healthcare decisions significantly.

The survey responses contained numerous ethical matters and the need for regulatory compliance. The group of postgraduate students and clinical practitioners agreed on the necessity of protecting patient information and maintaining proper ethical conduct [24].

The healthcare providers demonstrated misgivings because literature discussions address the need for artificial intelligence transparency, together with bias control and ethical monitoring.

Artificial Intelligence Potential in Oral and Maxillofacial Surgery: Opportunities for Transformation

The overall evidence indicates that artificial intelligence provides powerful opportunities to redefine Oral and maxillofacial surgery. The participants recognized multiple promising uses of artificial intelligence. These include: Artificial intelligence helps medical professionals achieve better results in image interpretation by checking radiographic and CT/MRI results to discover bone fractures, cysts, tumors, and infections [25]. Precision surgical guide and implant, and prosthesis development is enabled by integrating CAD/CAM systems, which implement Computer-Aided Design and Manufacturing technologies [26]. The employment of artificial intelligence-powered robotic systems is becoming more common in minimally invasive surgeries. RFID-tagged medical tools and gloves use smart technology to deliver interactive device feedback for ongoing surgical decisions and data recording.

Study Limitations

The research based its participant recruitment on a diverse number of individuals selected from the state of Kerala, India, but included the total participant count. The participants' responses through Google Forms demonstrate potential bias since they depend on both individual interpretation and the habit of offering socially acceptable responses [27]. The rigid design of the questionnaire restricts researchers from obtaining as many in-depth research findings.

Future Directions

These current results provide a solid basis for upcoming research ventures. Research following participants through time should measure how their views change concerning artificial intelligence, specifically before and after participating in artificial intelligence education for dental students. Educational

intervention studies about adding artificial intelligence modules to the dental curriculum and their effects on student success need to be implemented [22-23].

Practice-based artificial intelligence testing programs give medical professionals the opportunity to examine how these tools perform in actual patient procedures. Additional research should determine how dental professionals in orthodontics and periodontics, and prosthodontics view the implementation of artificial intelligence technology. This research shows that dental students and dental clinicians view artificial intelligence positively for oral and maxillofacial surgery practice, but their viewpoints remain affected by their handler experience and educational standing and clinical practice level. The extreme positive attitude toward artificial intelligence must overcome training gaps and infrastructure restrictions, along with moral problems, to succeed in practical adoption [24-27].

4.CONCLUSION

Artificial Intelligence now receives increased recognition for its ability to improve dental education and oral and maxillofacial surgery clinical practice, as this research confirms. Every participant agreed that artificial intelligence technology has beneficial effects on diagnostic accuracy and clinical support, and educational enhancement. The study shows that some differences exist in terms of artificial intelligence comprehension and preparedness for usage due to their varying practical hands-on experience. The study establishes a vital necessity to establish training programs to educate professionals about ethical artificial intelligence technology operations. The research provides baseline knowledge which researchers can build on to determine artificial intelligence's practical effects in dental practice.

AUTHOR CONTRIBUTIONS

S. Anil Kuzhivilayil: Conceptualization, Investigation, Writing original draft.
G. Skariah P: Methodology, Visualization, Supervision.
S. Dilna: Methodology, Visualization, Supervision.

5.DECLARATIONS

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ETHICAL APPROVAL

This study received ethical approval from the Ethics committee of PMS College Of Dental Science and Research Hospital under the protocol number PMS/IEC/2023/Additional/OCT/26.

INFORMED CONSENT

Written informed consent was obtained from all participants.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

COMPARATIVE STUDY BETWEEN SKIN GRAFTS AND FLAPS FOR THE TREATING NASAL DEFECTS AFTER BASAL CELL CARCINOMA EXCISION

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ABSTRACT

Background: The most prevalent type of skin cancer that affects the nose is basal cell carcinoma. The best treatment of this cancer is surgical excision with safety margin. After excision the defect that formed can be closed by skin grafts, locoregional flaps and microsurgery.

Aim of study: is to assess the outcomes of skin grafts and flaps used to repair nasal deformities following the removal of basal cell carcinoma from the nose.

Materials and Methods: This study was done in Al- Diwanyia teaching hospital and outpatient private clinic between January,2018 to April ,2024 for 36 patients were complaining from skin defect in the nose after(BCC) excision.The patients divided into two groups , each group consist of 18 patients.

The 1st group managed by skin graft and 2nd group by flaps.

In early follow up we assess the patients for presence of complications like infection ,flap ischemia , graft failure .We follow the patients for 6 months as a visit every month looking for late complications (scar problems ,pigment changes) and patient satisfaction .

Results: We found two patients developed wound infection those who manage by flap ,one patient developed tip nasolabial flap necrosis. Only one patients developed partial skin graft loss. 6 patients who treated by skin graft developed hyperpigmentation problems and this not occur in patients treated by flaps.

Conclusion: There are more advantages for using flap coverage in nasal reconstruction due to better cosmetic outcome and more patients satisfaction

Key Words: skin grafts, locoregional flaps, microsurgery, reconstructing nasal defects

INTRODUCTION

Nasal defects can results from trauma, burn or after tumors resection. In our study we focused on closure of the defects that resulted from basal cell carcinoma. Worldwide, the frequency of skin malignancies has increased; these diseases frequently affect the skin on the face.Most of the time, reconstruction is required once such face malignancies are removed [1]. The nose is the most projecting part of the face make it exposed more to sun light that lead to increasing the risk of skin cancers [2,3,4]. A nasal defect may affect the interior mucosal lining, the skin, cartilage, or bone. , therefore the surgeon who perform reconstruction of the nose should have good knowledge in surgical anatomy of the nose[5]. There are many option for closure nasal defects like skin grafts, locoregional flaps and microsurgery also play important role for total or near total nasal reconstruction6. Full thickness skin graft is well established for treatment of dorsum and sidewall defects of the nose because its flattened and thin skin that well recreated with skin graft only[7]. While other studies show that skin graft is an excellent reconstructive option for lower third nose because of its It is challenging to rotate a local flap into place due to its intrinsic thickness [8]. Some authors show skin graft less useful for lower third defects (alar and tip defects),which have established algorithms that often need flaps like v -y advancement flap ,nasolabial flaps and forehead reconstruction[9].

Patients and methods

This study compares the efficaciousness of skin grafts and flaps in reconstructing nasal defects following removal of basal cell carcinoma (BCC) from the nose.

following removal of basal cell carcinoma (BCC) from the nose.

This study was done in Al- Diwanyia teaching hospital and outpatient private clinic between January,2018 to April ,2024 for patients were complaining from skin defect in the nose after(BCC) excision.

Patient with disease comorbidities and patients with larger defects more than 3 cm were excluded from this study.

Thirty six patients were meeting the eligibility criteria. These patients were randomly divided into two groups, each group consist of 18 patients.

All the patients signed an informed consent form and the patients were studied clinically and evaluated before treatment according to sex, age and site of tumor.

All surgeries were done under local anesthesia that involve excision of the lesion with 0.5 cm safety margin and sent for histopathology.

All the results of histopathology were completely excised basal cell carcinoma.

With regard to the first group, a full thickness skin graft derived from the post-auricular area was used to close the defect that resulted from (BCC) excision.

The skin defects in the 2st group was closed by flaps, which included 12 v-y advancement flaps fig.1. and 6 patients treated by nasolabial flap fig. 2,3.



Figure 1.BCC sidewall of the nose treated by excision and v-y advancement flap



Figure 2. BCC right ala of the nose treated by excision and nasolabial flap

Figure 3. A nasolabial flap for closure defect in right ala after BCC excision. Picture was taken 6 months after surgery

The patients discharged home few hours post-operatively , at the same day of surgery.

In post-operative period, we give the patient oral antibiotic (Amoxicillin) with local antibiotic (fucidin) ointment and the sutures removed after 7 days post-operatively

In early follow up we assess the patients for presence of complications like infection ,flap ischemia , graft failure and hyperpigmentation .

We follow the patients for 6 months as a visit every month looking for late complications like scar problems and patient satisfaction .

A scale from 0 to 10 was used to measure the level of satisfaction (0 = no satisfaction, 1-4 = mild satisfaction, 5-7

= moderate happiness, 8-9 = satisfaction, 10 = great satisfaction).

The total number of patients involved in this study was 36 patients. 20 patients were male (56%) and 16 patients were female (44%). Table 1

Table 1. percent of gender number

Sex	Number	Percent
Male	20	56%
Female	16	44%
Total	36	100%

Age presentation ranged from 50 years to 85 years with mean age was 67 years.
The most common site affected by (BCC) is the ala nasi ,18 patients (50%) followed by sidewall ,13 patients (35%) and nasal bridge, 5 patients (15%) table 2.

Table 2. Site of BCC in the nose early follow up

Site	Number	Percent
Ala nasi	18	50%
Side wall	13	35%
Nasal bridge	5	15%
Total	36	100%

We found 2 patients developed wound infection those who manage by flap and treated by early removal of stiches, antibiotic and daily change dressing. Table .3
One patient developed tip nasolabial flap necrosis managed conservatively
Only one patients developed partial skin graft loss treated conservatively and this
not affected the final result fig 4.
6 patients who treated by skin graft developed hyperpigmentation problems and this not occur in patients treated by flaps

Table3. Early complications

Early complications	Skin graft	Flap
Wound infection	0	2
Necrosis	1	1
Hyperpigmentation	6	0

P value regarding early complications is significant (0.01)



Figure 4. Basal cell carcinoma treated by excision and skin graft and developed partial skin graft loss.

Late follow up
No scar problems was found in both groups.
Mean of patients satisfaction for patients treated by flap was 6.5±1.2 while for the patients treated by skin graft was 4.5 ± 1.4 (p value 0.001) .

DISCUSSION

The most prevalent type of skin cancer, basal cell carcinoma, is a malignant skin lesion that is destructive and locally invasive, and it seldom spreads. In our study we found more prevalence of male patients with nasal BCC but it is statically not significant, many studies reported male preponderance also this is due to prolong outdoor activities under the action of sun exposure [10].
The older population is more likely to develop basal cell carcinoma[11]. This finding is consistent with our study, as the majority of our patients are 67 years of age.Patients who are elderly are more likely to develop BCC due to factors such as weakened immune systems, diminished DNA repair capacity, and cumulative effects of prolonged sun exposure [12].
Reconstructing a nose with skin abnormalities might be difficult. Following the removal of nasal skin cancer, a number of reconstructive methods are available, such as skin grafting, free flaps, and local flaps.
The size, location, and involvement of deeper structures of the lesion all influence the reconstructive procedure that is chosen [13].
In our study we assessed 36 patients with nasal defects resulting from excision of BCC , these defects closed with skin graft or flaps.
In early follow up we found only one patient developed nasolabial flap tip necrosis and one patient developed partial skin graft loss , the 1st patient was heavy smoker and the 2nd was diabetic respectively, both treated conservatively and not affected the final results . We found there is significant difference regarding hyperpigmentation nwhen using skin graft this go with study was done by Jacob MA(14).
Contrary to our findings, some studies demonstrate that skin grafting provides a one-stage surgery with visually acceptable results that are comparable to those obtained from flaps [15].
When flaps were used, patient satisfaction was better; this could be because some patients who had skin grafting acquired postoperative hyperpigmentation. It was crucial that the recipient skin around the grafted area match in color [16].
Because donor site morbidity is reduced and broader skin grafts are possible, skin grafting is frequently used to cover significant skin defects following skin cancer resection [17].
We explain the outcomes and the result of both skin graft and flap for our patients
and we found more advantages for using flap coverage in nasal reconstruction due to better cosmetic outcome and more patients satisfaction. Reconstruction of nasal defects remains challenging due to different in size defect and complex three dimension of the nose

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

Informed Consent

Written informed consent were obtained from patients.

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ORIGINAL RESEARCH

ORAL GABAPENTIN AND LORAZEPAM AS PREMEDICATION IN PEDIATRIC ANESTHESIA: A RANDOMIZED COMPARATIVE STUDY

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ABSTRACT

Background: Preoperative anxiety in children can affect anesthetic induction, physiological stability, and postoperative outcomes. Lorazepam is a widely used benzodiazepine for anxiolysis, while gabapentin, although not commonly used in children, has shown potential anxiolytic effects.

Methods: This double-blind randomized controlled trial included 64 children aged 2–6 years who underwent elective procedures under general anesthesia. Subjects were randomly assigned to receive either oral gabapentin (15 mg/kg) or lorazepam (0.025 mg/kg). Preoperative anxiety was assessed using the Modified Yale Preoperative Anxiety Scale–Short Form (mYPAS-SF), while secondary outcomes included sedation, measured by the Ramsay Sedation Scale (RSS), and parental separation, assessed using the Parental Separation Anxiety Scale (PSAS).

Results: No significant reduction in anxiety scores was observed in either group (gabapentin: p = 0.29; lorazepam: p = 0.76). Optimal sedation occurred in 75% of lorazepam and 56.2% of gabapentin recipients. Inadequate separation and higher rescue sedation needs were more frequent in the gabapentin group. No moderate-to-severe adverse events were reported within 24 hours post-administration.

Conclusion: Neither drug significantly reduced preoperative anxiety scores, however, both demonstrated potential as part of pediatric premedication strategies. Gabapentin remains a promising alternative as premedication, particularly in specific clinical settings.

Keywords: Gabapentin; Lorazepam; Pediatric Anesthesia;Preoperative Anxiety Premedication

INTRODUCTION

Surgical procedures are among the most significant stressors experienced by hospitalized children and their families. In pediatric patients, this stress often manifests as heightened preoperative anxiety, which may lead to adverse postoperative behavioral changes. Studies have shown that the prevalence of preoperative anxiety up to 75% among children aged 2–12 years.[1]

Younger children, particularly those aged 2–6 years, tend to experience higher levels of anxiety. Other contributing factors include developmental status, temperament, prior surgical experience, and parental anxiety levels.[2]

Children may develop preoperative anxiety due to fear of medical staff, limited cognitive understanding, separation from parents, and needle-related procedures. This emotional stress triggers physiological responses such as increased heart rate, blood pressure, and excitability, resulting from sympathetic and parasympathetic activation.[3] High levels of anxiety have been associated with

prolonged recovery times, increased postoperative pain, delayed discharge, and behavioral disturbances

including sleep problems, enuresis, and separation anxiety.[4,5]

Reducing preoperative anxiety often involves pharmacological interventions, with benzodiazepines being commonly used.[6] Oral lorazepam, a long-acting benzodiazepine, has been considered a viable option for pediatric premedication due to its sedative and anxiolytic properties. However, benzodiazepines can occasionally cause paradoxical reactions, including agitation, hallucination, and disorientation, which may distress both the patient and their caregivers.[7]

Gabapentin, a second-generation anticonvulsant structurally similar to GABA, does not directly influence GABA metabolism but has shown promise in perioperative care.[8] It has been associated with reduced preoperative anxiety in both adults and children, blunted hemodynamic response to intubation, decreased postoperative nausea and vomiting, and reduced incidence of agitation and emergence delirium in pediatric populations.[9] However, evidence comparing gabapentin to standard anxiolytics like lorazepam in pediatric anesthesia remains scarce, especially in low-resource settings.

This study aims to compare the effects of oral gabapentin and oral lorazepam as premedication in pediatric patients undergoing elective surgery under general anesthesia, with a focus on preoperative anxiety reduction as the primary outcome.

METHODS

Study Design

This study was designed as a prospective, randomized, double-blind controlled trial conducted from Januari-March 2025 at Dr. Zainoel Abidin General Hospital, Banda Aceh, Indonesia. Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, Universitas Syiah Kuala (No. 1299/EA/FK-RSUDZA/2024), and the trial was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all parents or legal guardians of the participants.

Participants

Children aged 2–6 years scheduled for elective surgery under general anesthesia were screened for eligibility. Inclusion criteria were American Society of Anesthesiologists (ASA) physical status I or II, ability to take oral medication, and accompanied by a parent or guardian. Exclusion criteria included developmental delay, neurological or psychiatric disorders, known hypersensitivity to study drugs, recent use of sedatives or antiepileptics, and refusal to participate.

Randomization and Blinding

Participants were assigned to either the gabapentin group (G group) or the lorazepam group (L group) in a 1:1 ratio using systematic random sampling based on the order of subject arrival. Group allocation was conducted by a research assistant using a predetermined sequence and was not involved in the administration or evaluation process. The study medications had been previously prepared by the hospital pharmacy in powdered form and individually packed in identical, opaque, and sealed envelopes labelled only with subject codes. This method ensured blinding of both the anesthesiologist and the outcome assessor throughout the study.

Interventions

Children in the gabapentin group received oral gabapentin at a dose of 15 mg/kg body weight, while those in the lorazepam group received oral lorazepam at a dose of 0.025 mg/kg body weight. Both medications were administered at least two hours before anesthesia induction. Study drugs were prepared in powdered form by the hospital pharmacy and dispensed in identical sealed envelopes labelled according to subject codes. Intravenous cannulation was performed prior to premedication in all patients as part of the hospital's standard operating procedure, aiming to secure vascular access for potential emergency medication administration. Each child was accompanied by a parent or legal guardian up to the operating room to provide emotional support and minimize separation distress. If the child became uncooperative during monitor application or induction preparation, an additional sedative was administered at the discretion of the attending anaesthesiologist.

Outcomes

The primary outcome was the level of preoperative anxiety, assessed using the Modified Yale Preoperative Anxiety Scale–Short Form (mYPAS-SF), which was measured at two time points: prior to the administration of premedication and at least two hours after the medication was given. Secondary outcomes included sedation level assessed using the Ramsay Sedation Scale (RSS), parental separation measured by the Parental Separation Anxiety Scale (PSAS).

Sample Size

The required sample size for this study was calculated using a standard formula for comparative analytical research involving unpaired numerical data. The calculation was based on data from a previous study by Filho et al., assuming a moderate effect size, a significance level of 5%, and a power of 80%. The result indicated that a minimum of 26 subjects per group was required, yielding a total of 52 participants. To account for a potential dropout rate of 20%, the final target

sample size was increased to 62 participants.

Statistical Analysis

The distribution of preoperative anxiety scores measured by the Modified Yale Preoperative Anxiety Scale–Short Form (mYPAS-SF) was assessed for normality. Paired *t*-tests were used for within-group comparisons when data were normally distributed, while the Wilcoxon signed-rank test was applied for non-normally distributed data. Categorical variables, including sedation level based on the Ramsay Sedation Scale (RSS) and parental separation behavior assessed with the Parental Separation Anxiety Scale (PSAS), were analyzed using the Chi-square test. A two-sided *p*-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS software version 26.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Subject Characteristics

A total of 64 pediatric patients aged 2–6 years were enrolled in the study and randomized equally into the gabapentin group and lorazepam group (each n = 32). All participants completed the study and were included in the final analysis. Baseline characteristics such as sex and BMI were comparable between groups (table 1). However, there was a statistically significant difference in age distribution, with the mean age in the lorazepam group being higher than in the gabapentin group ($p < 0.05$). This difference was taken into consideration in the interpretation of outcomes.

Preoperative Anxiety (mYPAS-SF)

The level of preoperative anxiety was assessed using the Modified Yale Preoperative Anxiety Scale – Short Form (mYPAS-SF) before and after administration of premedication (table 2). In the gabapentin group, the mean anxiety score decreased from 44.9 ± 15.6 to 42.8 ± 13.1. however, this reduction was not statistically significant ($p = 0.29$). In the lorazepam group, the median anxiety score declined from 33.3 (IQR 16.7) to 31.3 (IQR 22.9), also without a statistically significant difference($p = 0.76$). These findings indicate that while both premedication showed a slight reduction in anxiety levels, neither produced a statistically significant change within their respective groups.

Sedation Level

The sedation level was evaluated using the Ramsay Sedation Scale (RSS), where a score of 2–3 was considered to indicate acceptable sedation. In the gabapentin group, acceptable sedation was achieved in 18 children (56.2%), while 14 children (43.8%) exhibited unacceptable sedation. In the lorazepam group, 24 children (75.0%) achieved acceptable sedation, whereas 8 children (25.0%) were assessed as unacceptable sedated. Although the proportion of acceptable sedation was higher in the lorazepam group, the difference between the two groups did not reach statistical significance ($p = 0.11$).

Parental Separation

Difficult separation from parents, indicated by a PSAS score of ≥ 3, occurred in 78.1% of children who received gabapentin and in 62.5% of those who received lorazepam. Although this difference was not statistically significant ($p = 0.174$), the findings suggest a higher tendency toward poor separation behaviour in the gabapentin group compared to the lorazepam group, implying a potentially more favorable effect of lorazepam on preoperative separation tolerance in pediatric patients.

Table 1. Demographic variables

Variables	Gabapentin	Lorazepam	p-value
Age (years) (mean ± SD)	3.78 ± 1.39	4.78 ± 1.43	0,006*
BMI kg/m2 (mean ± SD)	15.35 ± 3.80	14.71 ± 3.07	0,354
Sex (n)			
Male	15	21	0,208
Female	16	11	
Anesthesia History (n)			
Yes	6	13	0,055
Never	26	19	

Table 2. Comparison mYPAS-SF score before and after premedication

Group	Before	After	p-value
Gabapentin (Mean ± SD)	44,9±15,6	42,8±13,1	0,29
Lorazepam (Median(IQR))	33,3(16,7)	31,3(22,9)	0,76

Table 3. Comparison of sedation adequacy and parental separation between gabapentin and lorazepam groups

Variables	Gabapentin	Lorazepam	p-value
Sedation			
Acceptable sedation	18 (56,2%)	24 (75,0%)	0,11
Unacceptable sedation	14 (43,8%)	8 (25,0%)	
Parental Separation			
Acceptable separation	7 (21,9%)	12 (37,5%)	0,17
Difficult separation	25 (78,1%)	20 (62,5%)	

DISCUSSION

This study analysed baseline characteristics including age, body mass index (BMI), sex, and previous anesthesia history to ensure initial comparability between the gabapentin and lorazepam groups. A statistically significant difference was observed in the age variable, with the lorazepam group having a higher mean age (4.78 ± 1.43 years) compared to the gabapentin group (3.78 ± 1.39 years), p = 0.006. Body weight approached significance (p = 0.053), while no meaningful differences were found in BMI, sex distribution, or prior anesthesia exposure.

Age is a well-established predictor of preoperative anxiety in children. Children between 2 and 6 years old are particularly vulnerable to anxiety in the perioperative setting, representing a period of critical psychological development.[10] This age range includes two key phases: from 7 months to 3 years, where separation anxiety is dominant, and from 3 to 6 years, when fear of medical procedures becomes more pronounced. Children under 7 months rarely exhibit separation anxiety and are often soothed with physical comfort. However, toddlers and preschool-aged children may exhibit intense distress upon parental separation or when confronted with unfamiliar medical environments.[11]

In this study, younger children in the gabapentin group demonstrated significantly higher premedication anxiety scores than those in the lorazepam group.[2] Most children in the gabapentin group had scores above the clinical anxiety threshold, indicating moderate to severe anxiety prior to anesthesia induction. These findings support the notion that younger age is strongly correlated with higher preoperative anxiety risk. Therefore, the interpretation of anxiolytic effectiveness must consider the demographic imbalance—specifically the age difference—between the groups. This reinforces the importance of tailoring premedication strategies not only to pharmacologic efficacy but also to the child's developmental stage.

Premedication plays a pivotal role in facilitating a smooth transition to anesthesia, particularly in preschool-aged children who are highly susceptible to emotional distress in unfamiliar clinical settings. In this context, both pharmacologic and supportive non-pharmacologic interventions—such as simple

explanations, visualization tools, and mask desensitization—should be integrated to optimize patient comfort and minimize perioperative psychological trauma.

Preoperative anxiety in this study was assessed using the Modified Yale Preoperative Anxiety Scale–Short Form (mYPAS-SF). While both gabapentin and lorazepam groups showed slight reductions in anxiety scores following premedication, these changes were not statistically significant. Notably, all subjects had baseline anxiety scores above the clinical threshold (≥30), indicating a high prevalence of preoperative anxiety across the sample and suggesting limited anxiolytic efficacy from either intervention in this high-anxiety population.[12]

As shown in Table 2, both gabapentin and lorazepam groups demonstrated a reduction in anxiety scores after premedication; however, the changes were not statistically significant (gabapentin: $p = 0.29$; lorazepam: $p = 0.76$). All subjects had mYPAS-SF scores ≥30 before premedication, indicating a high baseline level of anxiety. While the gabapentin group showed a relatively consistent reduction in scores, the lorazepam group exhibited a wider post medication interquartile range despite a lower median score (from 16,7 to 22,9), suggesting greater variability in individual response. This may reflect differences in pharmacodynamic sensitivity or subtle paradoxical reactions, particularly relevant to benzodiazepine use in pediatric populations. The consistently elevated anxiety scores highlight the challenge of achieving adequate anxiolysis through pharmacological intervention alone. These findings emphasize the importance of incorporating non- pharmacological strategies alongside medication to optimize preoperative anxiety management in children.

The effectiveness of lorazepam observed in this study aligns with its pharmacological properties as a medium-acting benzodiazepine, which exerts anxiolytic effects by enhancing GABA- a receptor activity in the central nervous system.[13] With an onset time of 1.5 to 2 hours and a duration of 6 to 10 hours, lorazepam is considered suitable for pediatric use due to its lack of active metabolites.[6] However, the administration of a relatively low dose (0.025 mg/kg) in this study may have limited its maximal anxiolytic efficacy, suggesting that higher doses could be explored in future trials. A potential concern with benzodiazepine use in children is the risk of paradoxical reactions, such as agitation or restlessness, particularly in younger patients or those with neuropsychiatric susceptibility.[14] These effects are believed to be influenced by individual variability in receptor expression and neurotransmitter responses.[7] The wider interquartile range in the lorazepam group may indicate such heterogeneity in drug response, despite the absence of clinically apparent paradoxical effects during the observation period.

Gabapentin, initially developed as an anticonvulsant, has shown emerging promise as an anxiolytic in both adult and pediatric populations. Its mechanism of action does not involve direct modulation of GABA receptors; instead, it binds to the α2δ-1 subunit of presynaptic L-type calcium channels, leading to decreased release of excitatory neurotransmitters such as glutamate and substance P.[16] Downstream effects include reduced neuronal activity in limbic structures responsible for emotional regulation, as well as modulation of sympathetic tone via indirect inhibition of the *locus coeruleus*, a key brainstem region implicated in stress and arousal pathways.[17] These neurophysiological actions form the basis of gabapentin's anxiolytic potential, although its slower onset and less potent sedative profile relative to benzodiazepines may contribute to the more modest clinical outcomes observed in this study. Evidence from previous studies supports this pharmacodynamic profile. In a randomized trial comparing gabapentin at 15 mg/kg, 30 mg/kg, and placebo in pediatric surgical patients, both dosing regimens of gabapentin were associated with significantly lower mYPAS scores than placebo, indicating a dose-dependent anxiolytic effect.[18]

Several unmeasured factors may have influenced the outcomes observed in this study, particularly parental anxiety and the stress associated with intravenous (IV) cannulation. Parental anxiety has been shown to directly impact a child's emotional state, as children are highly perceptive of their parents' emotional cues—especially in high-stress situations such as surgical preparation. Excessive parental concern may unintentionally heighten a child's perception of threat, thereby amplifying their anxiety.[1,10] Furthermore, preoperative exposure to invasive procedures like IV insertion can serve as an additional stressor. Studies have demonstrated that unsedated IV placement may provoke strong emotional responses in children, increasing their risk of heightened preoperative anxiety.[1] In this study, IV cannulation was performed prior to the administration of premedication, potentially diminishing the observable anxiolytic effects of the drugs by exposing subjects to stressors before pharmacologic onset.

In pediatric anesthesia, a child's ability to achieve smooth separation from their parents and demonstrate readiness for anesthetic induction without the need for additional pharmacologic intervention is widely regarded as a key indicator of successful premedication. Among preschool- aged children, this separation phase is often the most emotionally

distressing component of the perioperative experience. It is strongly influenced not only by the pharmacological potency of the anxiolytic agent but also by the child's developmental maturity and psychological resilience. When adequate separation cannot be achieved, adjunctive sedatives—most commonly midazolam—are frequently employed to facilitate cooperation and ensure a safe and smooth induction process.

In the present study, as shown in Table 3, 78.1% of children in the gabapentin group and 62.5% in the lorazepam group experienced inadequate separation, as measured by the Parental Separation Anxiety Scale (PSAS). Although the difference was not statistically significant ($p = 0.17$), the trend suggests a potentially more favorable separation outcome associated with lorazepam. This finding, while preliminary, aligns with lorazepam's established pharmacological profile and highlights its potential utility in improving perioperative behavioral compliance.

The inability to achieve acceptable PSAS scores across both groups may be partly attributed to the conservative dosing regimens employed in this study. Lorazepam was administered at the lowest end of the recommended pediatric dosing spectrum (0.025 mg/kg), which may have limited its anxiolytic effectiveness in some children. Previous research has shown that oral midazolam at a dose of 0.5 mg/kg can reliably produce adequate separation in pediatric populations.[19] Furthermore, alternative agents such as nebulized ketamine and dexmedetomidine have demonstrated anxiolytic efficacy in similar clinical settings, though their use remains limited by regulatory approval in certain regions.[20]

Likewise, the gabapentin dose used in this study, although selected based on available pediatric data and safety considerations, may not have been sufficient to produce a clinically significant anxiolytic response. Given gabapentin's slower onset and different mechanism of action compared to benzodiazepines, its optimal anxiolytic dose and timing of administration remain subjects for future investigation. Refinement of these parameters-along with rigorous monitoring for adverse effects-may enhance its therapeutic value as a premedication agent and improve behavioral outcomes during parental separation.

In this study, the level of post medication sedation was assessed using the Ramsay Sedation Scale (RSS), a validated and widely utilized instrument for evaluating clinical sedation in perioperative care. As shown in Table 3, acceptable sedation—defined as an RSS score of 2 to 3— was achieved in 75.0% of patients in the lorazepam group compared to 56.2% in the gabapentin group. Although the difference was not statistically significant ($p = 0.11$), the results indicate a clinically relevant trend toward more consistent sedation with lorazepam. This aligns with its known pharmacological action as a benzodiazepine, enhancing GABA-A receptor activity in the central nervous system and producing a predictable sedative response within the two-hour post-administration window.[13]

In contrast, gabapentin is not a classical sedative and does not exert direct GABAergic activity. Instead, it binds to the α2δ subunit of voltage-gated calcium channels, reducing the release of excitatory neurotransmitters such as glutamate and substance P.[16] In addition to its anxiolytic and analgesic effects, gabapentin is believed to modulate noradrenergic tone through its indirect action on the locus coeruleus, a brainstem nucleus critically involved in arousal and stress responses.[17] By attenuating excitatory input within this region, gabapentin may contribute to a calming effect without inducing deep sedation.

This distinction has important clinical implications, particularly in pediatric anesthesia where airway safety is a priority. The relatively lower sedative profile of gabapentin may offer a safer pharmacological option for children at higher risk of respiratory compromise. While lorazepam may provide superior sedation efficacy, gabapentin's favorable safety margin may make it a viable alternative in clinical scenarios requiring anxiolysis without excessive sedation.

Limitations

This study has several limitations that warrant consideration. First, despite randomization, there was a significant difference in age distribution between the two groups, which could have influenced anxiety expression and drug response. Second, external psychosocial factors such as parental anxiety, child temperament, and prior exposure to the hospital environment were not controlled or measured, despite their well-documented impact on preoperative anxiety levels. Additionally, all patients underwent intravenous cannulation prior to drug administration, which may have acted as a procedural stressor and attenuated the anxiolytic effect of premedication, particularly in children with low distress tolerance. Finally, the assessment of adverse effects was limited to parental reports within 24 hours and did not include objective sedation-related respiratory outcomes, which may underestimate subtle or transient complications.

CONCLUSION

Oral gabapentin and lorazepam possess potential as pharmacological strategies for managing preoperative anxiety in pediatric patients. Although no statistically significant differences were observed in anxiety scores,

lorazepam consistently showed more favourable outcomes in sedation adequacy and separation parental. These findings suggest that lorazepam may be more clinically reliable in achieving the desired anxiolytic and sedative effects in the pediatric population. Nonetheless, gabapentin remains a promising alternative, particularly in specific clinical settings. Future studies should explore the integration of pharmacologic and non-pharmacologic approaches w hile accounting for psychosocial variables to optimize perioperative anxiety management in children.

DECLARATIONS

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Ethics approval

This study was approved by the Ethics Committee of The Ethics Committee of the Zainoel Abidin Hospital, in accordance with the Declaration of Helsinki and national ethical standards for research involving human subjects. The ethical clearance number is :311/ETIK-RSUDZA/2024, issued on 28th November 2024.

Written informed consent was obtained from all parents or legal guardians of participating children prior to inclusion in the study.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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COMPARISON BETWEEN STANDARD PROTOCOL AND GUIDED BIOFILM THERAPY IN PROFESSIONAL ORAL HYGIENE

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ABSTRACT

Background: Professional oral hygiene is pivotal in preventing periodontal disease by eliminating supragingival and subgingival biofilm and calculus. While traditional methods are effective, they may induce discomfort and tissue trauma. Guided Biofilm Therapy (GBT) is a minimally invasive protocol designed to optimize biofilm removal while improving patient comfort and operator ergonomics.

Aim: This study compares the Standard Protocol and GBT in terms of clinical efficacy, patient-reported comfort, treatment duration, and operator experience

Materials and Methods: The Present in vitro study utilized six subjects' blood and was centrifugated. T-PRF clots were prepared, and TI/ NE gel extracts were injected and compared with T-PRF alone to check the release of Vit C, Az, and IGF 1 at 6hours, 72 hours (3rd), 7th, and 14th day. For statistical analysis, a paired t-test and ANOVA were performed. P value <0.05 was considered statistically significant.

Results: Over 98% of patients treated with GBT reported reduced or absent pain compared to traditional methods. GBT reduced treatment time by approximately 15% and was associated with improved plaque control, reduced bleeding, and lower probing depths. Operators reported reduced fatigue and improved ergonomics with GBT instrumentation.

Conclusions: GBT offers a clinically effective and patient-friendly alternative to conventional prophylaxis, combining enhanced comfort with efficient biofilm management. These findings support the integration of GBT into routine oral hygiene practice to optimize clinical outcomes and patient compliance.

Key Words: GBT, scaling, professional hygiene

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1.INTRODUCTION

Periodontal disease is a multifactorial chronic inflammatory condition initiated by dysbiotic biofilm and influenced by systemic and local risk factors. Its effective management requires mechanical disruption of biofilm and patient adherence to preventive protocols. Traditional supragingival prophylaxis techniques—primarily manual or ultrasonic scaling—are often associated with discomfort, abrasiveness and variability in operator technique may limit their long-term efficacy and patient acceptance.[1-4] Guided Biofilm Therapy (GBT), developed by EMS, represents a structured, evidence-informed protocol to address these limitations. It integrates biofilm disclosing, air polishing with low-abrasive erythritol or glycine powders, selective use of ultrasonic devices, and patient education, all within a standardized eight-step workflow (1,5-7). This patient-friendly protocol emphasizes biofilm visualization and atraumatic debridement as central elements of modern periodontal maintenance. GBT is supported by recent literature demonstrating improvements in clinical outcomes and patient compliance. Clinical trials have shown reductions in bleeding on probing, plaque index, and gingival inflammation in patients treated with GBT compared to traditional methods[9-10]. Additionally, increased comfort and reduced procedural pain have been consistently reported, reinforcing its role in enhancing patient experience during hygiene sessions.

This study aims to assess the subjective response of patients undergoing GBT, with specific attention to comfort, pain, motivation, and overall satisfaction, using patient-reported outcome measures (PROMs).

2.MATERILAS AND METHODS

The findings aim to support the integration of GBT as a standard approach in prophylaxis, particularly for patients undergoing long-term periodontal maintenance.[11-12]



Figure 1. GBT technique

This comparative observational study enrolled adult patients in a professional oral hygiene recall program. Eligibility required good systemic health, the absence of periodontal conditions requiring surgical intervention, and informed consent. Patients were divided into two groups based on the hygiene protocol: the Standard Protocol group and the Guided Biofilm Therapy (GBT) group. The Standard Protocol included manual and ultrasonic scaling followed by rotary polishing using abrasive paste. The GBT protocol, in contrast, consisted of an eight-step sequence: medical history review, disclosing agent application for biofilm visualization, personalized hygiene instruction, air-polishing with erythritol powder via AirFlow®, subgingival instrumentation with PerioFlow® and Piezon® devices as needed, and final polishing. Each treatment was performed by experienced dental hygienists trained in both methods. Patient-reported experience was collected via a structured questionnaire assessing pain, comfort, noise level, and overall satisfaction using a 10-point Visual Analogue Scale (VAS). The operator recorded the treatment time. Ergonomic feedback was gathered through an operator-completed Likert scale regarding fatigue, posture, and instrument handling.Data were analyzed descriptively. Patient outcomes were compared qualitatively between groups, while ergonomic impact and efficiency were assessed from the operator's perspective.



Figure 2. Treatment with Guided Biofilm Therapy after plaque relief using colored relevant agents

3.RESULTS

The results of this observational study highlight a clear advantage of Guided Biofilm Therapy (GBT) over the Standard Protocol across all measured domains. Patients treated with GBT reported significantly lower pain levels, with an average VAS score of 1.8 (±0.6), compared to 5.8 (±0.9) in the control group. This substantial difference underscores the more atraumatic nature of the GBT approach.

In terms of perceived comfort during the procedure, GBT again outperformed the standard method. Patients rated their experience as markedly more comfortable, assigning an average score of 9.2 (±0.7) versus 6.5 (±1.1) for those treated with conventional scaling techniques. Similarly, noise sensitivity ratings favored GBT, with participants assigning it a mean score of 8.7 (±0.5), compared to 6.2 (±0.9) for the Standard Protocol, suggesting improved tolerability of the instrumentation used in GBT.

Overall treatment satisfaction was also higher in the GBT group, with an average score of 9.5 (±0.4) compared to 6.7 (±1.0) in the control group. Notably, a large majority—92%—of patients clearly preferred GBT and indicated they would choose it again for future sessions. This suggests that the subjective patient experience strongly favors the GBT approach.

Despite its structured workflow, the GBT protocol did not prolong treatment time. On the contrary, appointments were completed approximately 15% faster than with the standard protocol. This time efficiency likely results from the streamlined plaque removal process enabled by the AirFlow® system, which minimizes the need for prolonged mechanical instrumentation.

Lastly, the clinicians involved reported decreased physical strain and hand fatigue during GBT procedures.

4. DISCUSSION

The data showed that patients in the GBT group experienced significantly more favorable outcomes compared to the Standard Protocol group across multiple parameters. Pain levels reported during GBT were markedly lower, with a mean VAS pain score of 1.8, compared to 5.8 in the traditional group. The GBT approach also received higher ratings for comfort (mean 9.2 vs 6.5), reduced perceived noise (8.7 vs 6.2), and greater overall satisfaction (9.5 vs 6.7) (Figure 3).

Patient motivation appeared enhanced by the disclosing phase of GBT, with many respondents noting that biofilm visualization helped them understand oral hygiene priorities more clearly. In addition, 92% of patients preferred GBT over traditional scaling methods (Figure 4), citing improved tolerability and reduced post-operative discomfort.[13-15]

Despite a more structured and multi-step approach, Treatment time was reduced by 15% in the GBT group. This efficiency may be attributable to the faster biofilm removal capacity of the AirFlow® device compared to manual instrumentation.[16-20]

Ergonomically, hygienists consistently rated GBT as less physically demanding. The design of the AirFlow® handpiece and the minimized need for repetitive manual scaling translated into reduced musculoskeletal strain, improved posture maintenance, and decreased session fatigue. Operator satisfaction aligns with existing literature supporting the ergonomic benefits of air-polishing systems in dental hygiene settings.

These findings complement prior clinical studies demonstrating that GBT significantly reduces bleeding on probing and gingival inflammation, with fewer adverse tissue effects due to the gentle action of erythritol and glycine powders[21,22]. Although this study was observational in nature, the consistency in subjective measures supports GBT's implementation in maintenance care. [23-24]

The data collected in this observational study support the clinical and experiential superiority of Guided Biofilm Therapy (GBT) over the Standard Protocol for professional oral hygiene. Across all measured domains—pain perception, comfort, noise sensitivity, satisfaction, treatment efficiency, and operator ergonomics—GBT consistently yielded more favorable outcomes.

Patient-Reported Outcomes

Pain was significantly reduced in the GBT group, with a mean VAS score of 1.8, compared to 5.8 in the Standard Protocol group. This aligns with prior findings demonstrating that air-polishing with erythritol powder induces less soft tissue trauma than traditional ultrasonic scaling (16,19). Similarly, patients rated GBT higher in comfort (9.2 vs 6.5), noise tolerance (8.7 vs 6.2), and overall satisfaction (9.5 vs 6.7). These values reflect the GBT workflow's less invasive, more predictable, and patient-centric nature.

An additional strength of GBT lies in its visual component. The disclosing phase enhanced patient

motivation by providing immediate biofeedback on biofilm presence. This educational aspect fosters behavioral reinforcement, as previously noted by Furrer et al. [19], who reported improved home care adherence in patients exposed to biofilm visualization.

Furthermore, 92% of patients expressed a preference for GBT over conventional scaling, not only due to reduced procedural discomfort but also because of a lower incidence of post-treatment sensitivity and bleeding.

This subjective preference supports the notion that PROMs are critical in determining patient compliance and return rates for maintenance therapy [13–15].

Clinical Efficiency and Operator Experience

Despite its structured eight-step design, GBT showed a 15% reduction in treatment time, likely due to the enhanced efficiency of air-polishing devices in biofilm disruption. The AirFlow® system, in particular, has been demonstrated to remove supragingival and subgingival plaque more uniformly and rapidly than manual methods [16,20].

From an ergonomic perspective, GBT reduced perceived fatigue among hygienists. Thanks to the lightweight and ergonomic design of the GBT instrumentation, operators reported improved posture and less hand strain. These findings are consistent with literature suggesting that repetitive manual scaling contributes to musculoskeletal disorders among dental professionals and that air-polishing can mitigate this risk [21].

Comparison with Literature

The present results corroborate previous clinical studies showing GBT's effectiveness in reducing bleeding on probing and gingival inflammation, likely due to the anti-inflammatory effects of erythritol and the atraumatic application technique [11,17,21]. Additionally, GBT may reduce the cumulative trauma associated with repeated instrumentation, especially important in patients with exposed root surfaces, restorations, or dental implants [15,17,23]. In the broader context of oral hygiene protocols, the adjunctive use of chemical agents plays a fundamental role in enhancing the effectiveness of mechanical plaque removal. Among these, stannous fluoride has demonstrated significant potential in terms of enamel protection, antimicrobial activity, and reduction of gingival inflammation. A systematic review has recently summarized its multifactorial benefits, supporting its integration within routine oral hygiene regimens, particularly for patients undergoing scaling procedures or at high risk for caries and erosion. Furthermore, it should be acknowledged that the long-term success of periodontal and peri-implant maintenance protocols—particularly in patients rehabilitated with implant-supported prostheses—relies not only on professional debridement and oral hygiene measures, but also on patient satisfaction, compliance, and prosthetic stability. Recent clinical studies have investigated aspects such as the longevity of implant-retained overdentures and the application of fully digital workflows in implant-supported restorations, which can significantly influence plaque control and access to hygiene procedures in the long term. Moreover, the prevention and management of enamel demineralization phenomena, such as white spot lesions, represent a critical endpoint in the continuum of care related to oral hygiene. These lesions often result from inadequate plaque control and are frequently observed in patients undergoing orthodontic treatment or those with poor compliance with oral hygiene instructions. Recent systematic reviews have emphasized the importance of early detection and the integration of non-invasive therapeutic strategies as part of a comprehensive hygiene protocol. In the broader context of emerging biofilm control strategies, recent investigations have highlighted the potential of adjunctive therapies such as ozonated olive oil in modulating oral microbiota, reducing inflammation, and enhancing mucosal healing. These approaches align with minimally invasive, patient-centered dentistry principles and may complement mechanical debridement methods in selected clinical scenarios [24-29].

While the study's observational nature precludes definitive causal inferences, aligning subjective outcomes with GBT's mechanistic advantages lends credibility to its implementation in maintenance protocols.

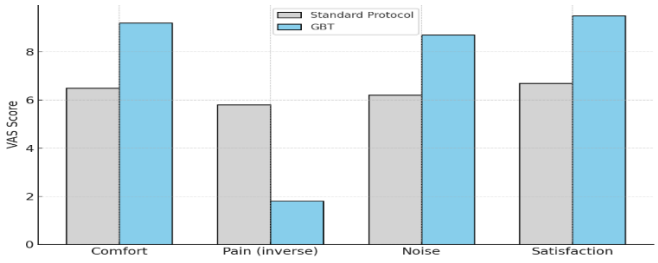


Figure 3. Comparison of mean VAS scores (0–10) across four categories: comfort, pain (inverted scale), noise perception, and overall satisfaction between GBT and Standard Protocol groups.

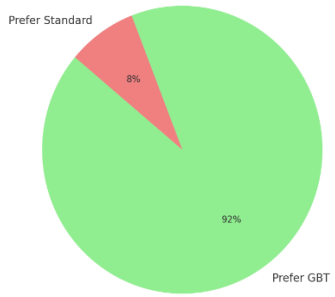


Figure 4. Distribution of patient preference between Guided Biofilm Therapy and the traditional standard protocol after undergoing hygiene treatment.

4.CONCLUSION

This observational study confirms that Guided Biofilm Therapy (GBT) provides a superior patient experience and greater clinical efficiency compared to the standard protocol for professional oral hygiene. The statistically and clinically significant differences in pain perception, comfort, satisfaction, and treatment time highlight GBT as a more favorable approach from both the patient's and operator's perspective.

Patients treated with GBT consistently reported lower discomfort and greater tolerance to instrumentation and noise. The visual disclosing phase and use of minimally invasive devices contributed to increased patient engagement and adherence, elements that are often overlooked in traditional hygiene protocols. The high preference rate for GBT suggests that patient-reported outcomes should be more routinely considered in evaluating maintenance strategies.

In addition, the ergonomic benefits observed by the clinical staff, such as reduced hand fatigue and shorter appointment duration, suggest that GBT may contribute to long-term occupational health among oral health professionals.

Clinical Recommendations

- GBT should be considered the standard of care in periodontal maintenance, especially in patients with dentinal sensitivity, restorative work, or implant-supported prostheses.
- Dental hygienists and clinicians should be trained in GBT to ensure protocol fidelity and maximize its ergonomic and biological benefits.
- Future studies should aim to validate these findings in randomized controlled trials with longer follow-up and clinical endpoints such as bleeding on probing, plaque index, and probing depth.

Incorporating PROMs as a routine measure of treatment success is strongly recommended, as they capture dimensions of care quality that go beyond mechanical biofilm removal and directly impact patient loyalty, motivation, and health outcomes.

DECLARATIONS

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Conflicts of interest and financial disclosures

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Ethical approval

Approval for the conduction of the study was obtained from the Institutional Scientific Review Board (SRB/SDC/PhD/PERIO-2251/25/025).

Informed Consent

Verbal and written informed consent were obtained from healthy volunteers

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COMPARATIVE EVALUATION OF AZADIRACHTIN, VITAMIN C AND INSULIN LIKE GROWTH FACTOR 1 RELEASE IN TITANIUM PLATELET RICH FIBRIN INFUSED WITH NEEM AND TRIPHALA INDICA GEL EXTRACTS: AN INVITRO STUDY

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ABSTRACT

Background: Herbal extract gel addition to Titanium -Platelet Rich Fibrin (T-PRF) clots was started very recently. Their release from the T-PRF clots has not been performed. Hence present study aimed to evaluate the release of Azadirachtin (Az) from T-PRF+Neem gel group, Vitamin C release from T- PRF+ Triphala Indica group and Insulin like growth factor 1 (IGF 1) from T-PRF alone along with the mentioned test groups.

Methods and Materials: Present invitro study utilized 6 subject's blood and subjected to centrifugation. T-PRF clots were prepared and TI/ NE gel extracts were injected and compared with T-PRF alone to check the release of Vit C, Az and IGF 1 release at 6hours, 72 hours (3rd), 7th and 14th day. For statistical analysis, paired t test and ANOVA was performed. P value <0.05 was considered statistically significant.

Results: Az release there was decreased levels reported between 6 hrs and the 7th day and it was statistically significant (p = 0.045*). Whereas, all other time frame comparisons values were non-significant (p>0.05) Regarding Vit C release from T-PRF clot there was a decrease in the amount released when compared at 6hrs & 7th day (p=0.008*) and 72hrs and 7th day (p=0.037*). IGF 1 release in all the three groups and at different time frames was non-significant (p>0.05).

Conclusion: Added herbal extracts didn't alter the release of IGF 1 that proves the surety of GF release. T-PRF also showed the timely release of the Az and Vit C confirming the name of sustained drug delivery system (SDDS).

Key Words: Azadirachtin, Ascorbic Acid, Insulin like Growth Factor, Platelet Rich Fibrin, Periodontitis, Titanium

INTRODUCTION

Systemic antibiotics application has been a practice since ages for the control of infection pre and post treatments.[1] With the extensive usage of these medicines there was a development of side effects which is very difficult for doctors, researchers and patients to tackle the disease entity patterns and routes. Apart from this, there was development of resistance for bacteria, side effects to living beings led the researchers to develop newer delivery vehicles to release the drug at constant pace.[2] Materials like carbopolymer, polaxamer 123, collagen membranes, collagen plugs, microspheres etc have been used to deliver the drugs. But because of their increased incidence of antigenicity better biomaterials were searched.[3]

They came across platelet concentrates (PC) which were autologous and had a greater sustained capability of holding growth factors. They also had a three-dimensional fibrin structure which also hold platelets, white and red blood cells.[4] First generation PC like Platelet rich Plasma, Fibrin glue and Second-generation PC like Leukocyte Platelet Rich Fibrin (L-PRF), Advanced Platelet Rich Fibrin (A-PRF) were used regularly in different treatment strategies such as intra bony defects (IBD), gingival recession treatments, sinus augmentation procedures with and without adding bone graft materials.[5] Miron RJ et al.,[6] 2021 in their systematic review and meta-analysis concluded that PC's addition along with bone grafts helped in better treatment outcomes such as reduction in probing pocket depth, gain in CAL and improved bone fill and alveolar crest change. Hence, they have used this PRF for delivering the drug either antibiotics or anti-inflammatory drugs to tackle the post operative pain and infection.[7] Study done by Pollock D et al.,[8] 2019 utilized metronidazole, penicillin and clindamycin in the L-PRF and checked for antimicrobial efficacy for staphylococcus aureus and fusobacterium nucleatum where they concluded that PRF incorporation of antibiotics helped in greater antimicrobial efficacy than L-PRF alone. They also stated that 0.5ml drug injection into the L-PRF didn't alter the structural properties of it. Beyond that there was abnormalities noted for PRF structure. Pillai AK et al.,[9] 2021 incorporated diclofenac sodium pain killer into L-PRF and placed at the 3rd molar extraction sockets where they reported that test sites reported better than control group with reduced post-operative pain. Healing was also better in test sites.

But because of raised concerns regarding the possible silica contamination[10] in silica coated plastic tubes, breakage of tubes made of silica and shorter resorption time led to the search of better material that can be used.[11] This led to the introduction of Titanium Platelet Rich Fibrin (T-PRF) by Tunali M et al.,[12] 2013

where they prepared this in grade IV titanium tubes. They concluded that Titanium tubes had better haemo compatibility, titanium dioxide passivates itself within the tubes and activate the platelets similar to silica in glass tubes.[13] The fibrin structure that formed was thicker, denser, with superior fibrin border area. This was supported by later studies done by Chatterjee A et al.,[14] 2017, Mitra DK et al.,[15] 2019, Bhattacharya HS et al.,[16, 17] 2020 & 2022 where they concluded that T-PRF had better fibrin meshwork, superior cellular entrapment and better hold of growth factors than L-PRF. Tunali M et al.,[18] 2014 also concluded that T-PRF had a longer resorption time of 21 days when checked with rabbit's study. With this evidence, clinical trials regarding hard and soft tissue parameters were performed and achieved good results regarding the healing and improvement of PPD, CAL, bone fill, increased keratinized tissue width, improved mean root coverage and reduced recession depth & width.[19-21] Hence, Ercan E et al.,[22] 2022 used this T-PRF as sustained drug delivery system (SDDS) where they added doxycycline hydrochloride solution into T-PRF and studied regarding the drug kinetics where they concluded that T-PRF has a better with holding capacity and timely release of drug. They also showed good antimicrobial property against the staph aureus and pseudomonas aeruginosa bacteria. Recent study done by Gummaluri S S et al.,[23, 24] 2024, 2025 incorporated amoxicillin+ clavulanate gel, metronidazole and neem gels into T-PRF and checked for fibrin mesh work border and cellular entrapment. They concluded that T-PRF structure didn't alter and had a similar structure to that of T-PRF alone when checked for light and scanning electron microscopies.

With the increased hypersensitivity and resistance of antibiotics there was a gradual shift into herbal extracts. In the recent trends their utilization had gradually increased in the form of mouth washes, gels for topical applications in gingivitis and periodontitis condition. Extracts such as neem, aloe-vera, mangosteen, curcumin, Triphala indica, Matricharia chamomile, green tea catechins etc have been used as an adjunct therapy in treating gingival and periodontal diseases. Most of these extracts exhibit anti-oxidant, anti-inflammatory, antimicrobial, anti- carcinogenic, anti-cariogenic properties and help in reducing the bacterial load, post-operative inflammation which in turn improve the health of the tissues.[25] Studying the release of the components from the main drug at regular time interval, help in identifying the efficacious nature of the drug that would be released. This would enhance the development of newer treatment strategies. In the present study, there was

a utilization of Neem and Triphala indica extracts in the form of gels and incorporated into T-PRF.

Triphala, a blend of three plants consists of carotene, D-glucose and fructose, riboflavin empicol, chebulinic acid, vitamin C, arachidonic acid, linoleic acid, oleic, palmitic, and stearic acid, tannic acid, terchebin, and other essential components. These components help control cell death and lower lipid peroxidase activity, which indicates the antioxidant, anti-inflammatory, antimicrobial, and antiseptic qualities of TI.[26, 27] Whereas Neem consists of Azadirachtin, nimbin, nimbidin and some additional components such as flavonoids, alkaloids etc. showing antiseptic, insecticidal, antiulcer, astringent etc.[28] Studies were performed to check the efficacy of these herbal extracts on gingivitis or periodontitis conditions as topical application or as LDD and reported good results.[29, 30] These were not tried as SDDS by incorporating into T-PRF. Before performing a human trial, it is always beneficial to perform invitro substance release so that the biomaterial integrity and GF release hampering will be known. Study was performed according to null hypothesis that adding the Neem and Triphala gels to T-PRF clots will alter the release of Insulin like growth factor 1 and there will be no timely release of azadirachtin and vitamin C through the T-PRF clots. Hence present study aimed to evaluate the release of Azadirachtin (Az), Vitamin C (Vit C) and Insulin like Growth Factor 1 (IGF 1) release from T-PRF clots infused with Neem and Triphala indica gels to check T-PRF as sustained drug delivery system (SDDS).

MATERIALS AND METHOD

Study design and Patient Recruitment

Present study was a primitive invitro study where IGF 1, Vit C and Az releases were checked at various time frames. A total of 6 healthy (mean age of 26.4 years) were recruited in the study 3 males and 3 females from the out-patient department of periodontics Saveetha dental college and hospital, Chennai. Study was performed during the tenure of April 1st to May 10th 2025. Oral and written informed consent was obtained from the volunteers. Before performing the study, approval from the scientific review board was obtained and approval number as SRB/SDC/PhD/PERIO-2251/25/025. Study was performed according to the CRIS guidelines that report the invitro studies. Patient blood was drawn hence Helsinki declaration 1975 was also followed to maintain the reliability of the study and respecting the volunteers.

Inclusion and Exclusion Criteria

Subjects who were voluntarily willing to participate having greater than 2,00,000 platelets per cubic millimeters, not having any systemic disease, non-smokers, not under any sought of medication that would affect the blood and blood products were included in the study. Subjects who were not interested, pregnant and lactating females, any subjects that reported with some ailments after blood investigation were excluded from the study.

Herbal Extract gel Preparations:

Neem (NE) and Triphala indica (TI) Gels were procured from Perio Biologics ™ Lab, Hyderabad, Telangana, India. Briefly gels were prepared as follows- TI is a combination of three fruits Terminalia chebula (Haritaki), Terminalia bellrica (Bibhitaki) and Emblica officinalis (Amalaki). TI power is mixed with distilled water and left for few hours. Later mixture was strained to obtain a clear extract. Further this prepared extract was mixed with hydroxyethyl cellulose to obtain a homogenous mixture. glycerin & phenoxyethanol were added and pH was adjusted with proper stirring to obtain a final 22%w/v concentration of TI gel. While coming to neem gel preparation, neem leaves were boiled to prepared concentrated neem extract. Carbopol 943p was added to the neem extract to prepare the base gel. To this extract calcium chloride ($CaCl_2$) and Sodium Chloride (NaCl) were added to prepare the final 12% w/v concentrated NE gel. Both the gels were packed into syringes, subjected to stability testing and properly sterilized to use it further. All the raw materials were obtained from local ayurvedic shop vendors. Additional binder and gel preparation materials were procured from Sigma Aldrich Company.[31, 32]

PROCEDURE

T-PRF clots Preparation and herbal extracts injection

Based on Modified Tunali M et al., protocol of 3500rpm for 15 min with 400 x *g* force, blood of 15ml was drawn from antecubital vein (divided into 5 ml each) and transferred to medical grade titanium test tubes (Supra Alloys Company Camarillo USA) without any anticoagulant and subjected to centrifugation using (Remi R 8C, New Delhi, India). Clots were retrieved using sterile tweezers and transferred into kidney trays. Hence, a total of 18 T-PRF clots were retrieved and divided in to 3 groups each containing 6 clots (T-PRF+NE, T-PRF+TI and T-PRF alone. Prior to conduction of experiment, herbal extracts (NE and Az) were injected into the clots individually based on Ercan E et al.,[22] 2022, Gummaluri SS et al.,[23, 24] 2024 and 2025 protocol where gels were injected directly into clots without compressing them into membranes. Later these clots were macerated and that liquid was stored at -70°C. Further, at every time point 5ml of that macerated clot fluid from test pool and subjected to analysis

Regarding Azadirachtin release from T-PRF infused with NE, all the clots were macerated and approximately 5ml of the liquid was mixed (Figure 1) and shaken for 10-20 minutes with 5ml of HPLC grade methanol. The solution was then

filtered through 0.45 µm membrane filter. Accurately measured 1ml from above solution was further diluted with 10 ml with methanol. Accurately measured 4.0 mL portion of Azadirachtin extract solution was diluted to 10 mL with methanol so as to prepare concentration of 40 µg/ml. Equal volume (40 µL) of standard and sample solutions was injected separately after equilibrium of stationary phase. The HPLC method for estimating azadirachtin levels was used which involves reverse-phase chromatography with a C18 analytical column and a methanol-water mobile phase. The detection was done using a UV detector (HPLC©, Acidum India Pvt Ltd, Pune, India) at 222 nm, and the flow rate is maintained at 1 mL/min.[33]

While coming to Vit C release from T-PRF infused TI similar to the above procedure All the clots were macerated and approximately 5ml of the liquid was mixed and shaken for 10-20 minutes with 5ml of HPLC grade methanol. The solution was then filtered through 0.45 µm membrane filter. Reverse-phase C18 column is commonly used. Tissue fluids were deproteinized using metaphosphoric acid before injection into the HPLC system (HPLC©, Acidum India Pvt Ltd, Pune, India) and detection of Vit C was performed at 254 nm.[34] Though we used HPLC, the results were compared with a standard Vit c kit, Ascorbic Acid Test Kit, Model ASC-1, Hach India, New Delhi (Figure 2 and 3). Regarding IGF 1 release enzyme linked immunosorbent assay (ELISA) test (Miltenyi Biotec, Inc, Auburn, CA, USA) was used and performed according to manufacture instructions. The sensitivity and evaluation range for parameter was- human IGF- 1:(0.94 ng/ml & 1.56-100ng/ml). At time frames of 6 hours, 72 hours, 7th and 14th day, Vit C (µmol/L); Az (µg/ml) and IGF 1 (ng/ml) release was checked. Further data was retrieved using ELISA plate reader (for IGF 1), UV detectors were used for Vit C and Az release. Absorbance values were calculated and release output was tabulated into the Microsoft excel spreadsheet.

STATISTICAL ANALYSIS

Using statistical package for social sciences (SPSS) version 22 IBM Pvt Ltd, Chicago, Illinois, USA, study analysis was performed. Data were expressed in mean and standard deviations. Paired t test was performed to check the time wise comparisons for T-PRF+NE (Az release) and T-PRF+TI (Vit C) release. For IGF 1 levels assessment for T-PRF+NE, T-PRF+ Az and T-PRF alone groups paired T-Test was used to check for time variations. Whereas one way ANOVA was used for IGF 1 level comparison for all the three groups at different time frames of 6hrs, 72 hrs, 7th and 14th day. A p value of <0.05 was considered statistically significant.



Figure 1: Depict the Neem Test Extract solution used in the present study



Figure 2. Depict the Vitamin Reference Solution used in the study



Figure 3. Depict the image of Vitamin C kit used in the study

Table 1. Depict the comparative analysis of Azadirachtin levels in T-PRF + Neem at different time intervals.

		T-PRF + Neem			
TIME		**Azadirachtin µg/mL**			
		Mean ± SD	**Mean ± SD**	**mean Difference**	**P-Value**
6hrs	**72hrs**	42.37 ± 5.93	43.35 ± 9.28	-0.98	0.870#
6hrs	**7th DAY**	42.37 ± 5.93	38.12 ± 6.05	4.24	0.045*
6hrs	**14th DAY**	42.37 ± 5.93	44.12 ± 7.79	-1.75	0.722#
72hrs	**7th DAY**	43.35 ± 9.28	38.12 ± 6.05	5.23	0.440#
72hrs	**14th DAY**	43.35 ± 9.28	44.12 ± 7.79	-0.77	0.829#
7th DAY	**14th DAY**	38.12 ± 6.05	44.12 ± 7.79	-5.99	0.273#
TIME		**IGF1 (ng/mL)**			
		Mean ± SD	**Mean ±SD**	**mean Difference**	**P-Value**
6hrs	**72hrs**	15.58 ± 2.44	15.28 ± 2.95	0.31	0.787#
6hrs	**7th DAY**	15.58 ± 2.44	15.48 ± 2.87	0.11	0.948#
6hrs	**14th DAY**	15.58 ± 2.44	14.95 ± 2.33	0.63	0.686#
72hrs	**7th DAY**	15.28 ± 2.95	15.48 ± 2.87	-0.20	0.882#
72hrs	**14th DAY**	15.28 ± 2.96	14.95 ± 2.33	0.33	0.801#
7th DAY	**14th DAY**	15.48 ± 2.87	14.95 ± 2.33	0.53	0.640#

SD- Standard Deviation; µg- micro gram, mL- milli liters, ng-nano gram, * indicates statistical significance, # indicate non-significant, $p<0.05$ considered statistically significant

RESULTS

Regarding the Azadirachtin (Az) release there was decreased levels reported between 6 hrs and the 7th day and it was statistically significant ($p = 0.045$*). Whereas, all other time frame comparisons values were non-significant ($p>0.05$) indicating the stable release of Az. Further, when IGF 1 levels were assessed all the comparisons were non-significant (Table 1). Regarding Vit C release from T-PRF clot there was a decrease in the amount released when compared at 6hrs & 7^{th} day ($p=0.008$*) and 72hrs and 7^{th} day ($p=0.037$*). There was a marked significant reported from 7^{th} to 14 day which was statistically significant ($p=0.006$*) while remaining comparisons were non-significant ($p>0.05$). Further, when compared for IGF 1 release at different time frames they were non-significant (Table 2).

In T-PRF alone group there was non-significant release of the IGF 1 at all the time frames (Table 3). When overall comparisons were performed in all the three groups and at different time frames, IGF 1 release report was non-significant (Table 4 and Graph 1).

Table 2. Depict the Analysis of Vitamin C Levels in T-PRF + Triphala at different time intervals.

TIME		T-PRF+ Triphala Vit C µmol/l			
		Mean ± SD	Mean ± SD	mean Difference	P-Value
6hrs	72hrs	180.17 ± 21.71	172.83 ± 28.94	7.33	**0.191**#
6hrs	7th DAY	180.17 ± 21.71	151.28 ± 18.39	28.89	**0.008***
6hrs	14th DAY	180.17 ± 21.71	178.15 ± 15.92	2.01	**0.855**#
72hrs	7th DAY	172.83 ± 28.94	151.28 ± 18.39	21.56	**0.037***
72hrs	14th DAY	172.83 ± 28.94	178.15 ± 15.92	-5.32	**0.681**#
7th DAY	14th DAY	151.28 ± 18.39	178.15 ± 15.92	-26.88	**0.006***
TIME		T-PRF+ Triphala IGF1 (ng/mL)			
		Mean±SD	Mean± SD	mean Difference	P-Value
6hrs	72hrs	14.69 ± 2.25	15.49 ± 2.31	-0.80	**0.377**#
6hrs	7th DAY	14.69 ± 2.25	14.87 ± 2.64	-0.18	**0.636**#
6hrs	14th DAY	14.69 ± 2.25	16.35 ± 2.35	-1.66	**0.236**#
72hrs	7th DAY	15.49 ± 2.31	14.87 ± 2.64	0.62	**0.392**#
72hrs	14th DAY	15.49 ± 2.31	16.35 ± 2.35	-0.86	**0.428**#
7th DAY	14th DAY	14.87 ± 2.64	16.35 ± 2.35	-1.48	**0.264**#

SD- Standard Deviation; µg- micro gram, mL- milli liters, ng-nano gram, * indicates statistical significance, # indicate non-significant, p<0.05 considered statistically significant

Table 3. Depict the analysis of IGF1 Levels in T-PRF alone at different time intervals.

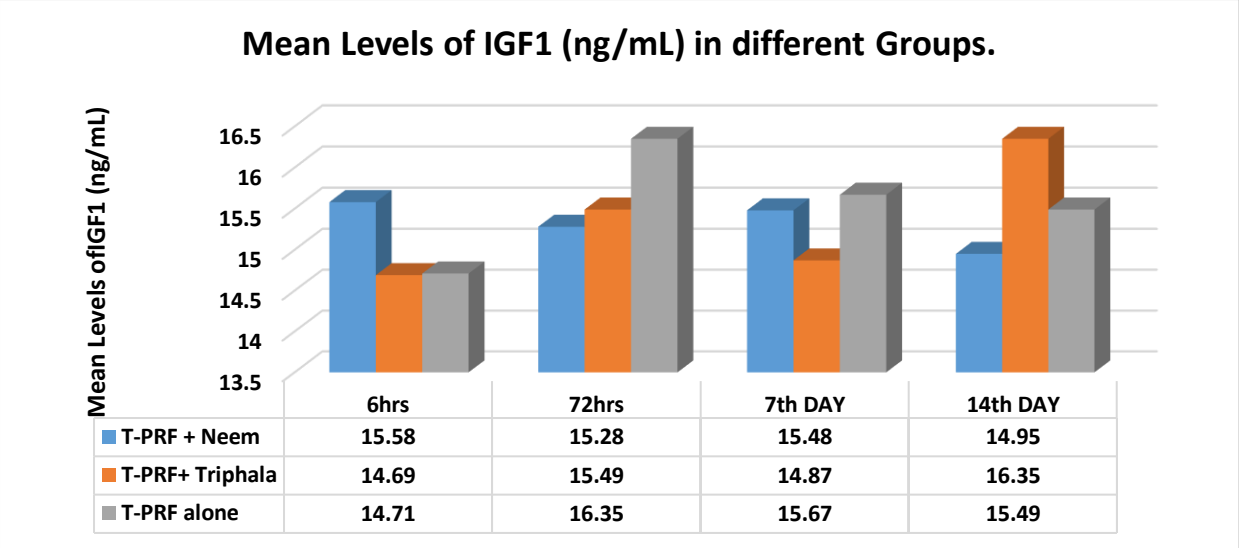
TIME		T-PRF alone IGF1 (ng/mL)			
		Mean ± SD	Mean ±SD	mean Difference	P-Value
6hrs	72hrs	14.71 ± 1.75	16.35 ± 2.06	-1.64	**0.305**#
6hrs	7th DAY	14.71 ± 1.75	15.67 ± 2.08	-0.96	**0.511**#
6hrs	14th DAY	14.71 ± 1.75	15.49 ± 1.39	-0.78	**0.488**#
72hrs	7th DAY	16.35 ± 2.06	15.67 ± 2.08	0.68	**0.328**#
72hrs	14th DAY	16.35 ± 2.06	15.49 ± 1.39	0.86	**0.319**#
7th DAY	14th DAY	15.67 ± 2.08	15.49 ± 1.39	0.18	**0.803**#

SD- Standard Deviation; µg- micro gram, mL- milli liters, ng-nano gram, * indicates statistical significance, # indicate non-significant, p<0.05 considered statistically significant

Table 4. Depict the comparison of IGF1 Across All Groups at Different Time Intervals using One-way ANOVA test.

	IGF1 (ng/mL)				
	T-PRF + Neem	T-PRF+ Triphala	T-PRF alone		
TIME	Mean±SD	Mean±SD	Mean±SD	F-Value	P-Value
6hrs	15.58 ± 2.44	14.69 ± 2.25	14.71 ± 1.75	0.334	0.722#
72hrs	15.28 ± 2.95	15.49 ± 2.31	16.35 ± 2.06	0.317	0.733#
7th DAY	15.48 ± 2.87	14.87 ± 2.64	15.67 ± 2.08	0.161	0.853#
14th DAY	14.95 ± 2.33	16.35 ± 2.35	15.49 ± 1.39	0.698	0.513#

IGF- insulin like growth factor, SD- standard deviation, # indicates non- significant



Graph 1 Depicts the overall mean levels of IGF 1 at different time frames for all the three groups

DISCUSSION

Present invitro study was performed mainly to assesses whether injecting TI and NE will hamper the release of growth factor from T-PRF and vice versa i.e. whether T-PRF will hold the extracts and release them timely. Vit C, Azadirachtin (Az) and IGF 1 release were checked was mainly because Vit C helps in proper wound healing and very much important for post translational modifications of proline and lysine proteins.[35] Az has antibacterial antifungal and anti-inflammatory properties that would combat the periodontitis causing bacteria.[28] Reason for assessing IGF 1 was mainly due to check whether apoptotic effect was happening due to the addition of extracts. Moreover, IGF 1 helps in wound healing, repair of soft and hard tissues by stimulating the cell proliferation and differentiation.[36] To the authors knowledge much number of studies were not performed for comparing the present study results. Hence existing other substance literature was compared to depict the present study results. Present study results were in accordance with study done by Pollock D et al.,[8] 2019 where they checked the incorporation of antibiotics in L-PRF helped in better outcomes regarding the antimicrobial efficacy and reduced post-operative infection. In the present study these herbal extracts (Az/ Vit C) release was also timely and didn't hamper the release of IGF 1 from T-PRF which indirectly indicate that T-PRF can be used as a SDDS. In Ercan E et al.,[22] 2022 study T-PRF clot timely released doxycycline antibiotic up to 7 days and also showed good antimicrobial property whereas similar release of Az and Vit C was reported from T-PRF was reported up to 14 days. This release will be helping to maintain good healing property (Vit C and IGF 1 release not hampered) as well as antimicrobial property (Az has anti-inflammatory and antimicrobial property) reducing the load of microbiota when explored in clinical studies.

The timely release of Vit C helps in good wound healing and better anti-inflammatory property which would enhance the treated site positively when used clinically. Thus, in the present study there was a check for Vit C release up to 14 days. This was supported by a systematic review done by Katariya C and Jayakumar N D[37] 2022 where they concluded that Vit C helped in improvement in clinical parameters such as probing pocket depth (PPD), clinical attachment level (CAL). It also activates the collagen synthesis by stimulating the procollagen m RNA and collagen transcription mechanism.

Study such as Ganvir MN et al.,[38] 2022 stated the use of Az chip (Neem chip) and Laser adjunct to non-surgical periodontal therapy (NSPT) they concluded that Az chip had shown better improvement in clinical parameters followed by laser and least by NSPT alone during the 1 month follow up. While coming to Heiman L et al.,[39] 2017 stated that neem leaf extract had a greater antioxidant property as well as antimicrobial property against Porphyromonas gingivalis (Pg) when interacted with red blood cells, lysozyme and bacteria. Hence, present study results may be comparable and confirmable as it showed the in-vitro release of Az from T-PRF up to 14th day which may be beneficial in the control of subgingival microbiota through improved anti-oxidant property. This also show the anti-inflammatory property. Regarding IGF 1 release there was no significant difference in all the groups at all the time frames this is in accordance with study done by Fukui N et al.,[40] 2015 where they stated that there no significance regarding the release of IGF 1 for L-PRF. They stated that IGF 1 is a multifunctional peptide that helps in osteogenic

differentiation of bone marrow mesenchymal stem cells (BMSC's) and human dental pulp stem cells (HDPSC's).[41] The levels of IGF 1 in the reported study was much higher than current study and this might be due to the amount of blood that was drawn for clot preparation varied. They also stated that levels of IGF 1 were almost similar to that of human blood.

Limitations of the present study might be smaller sample size and invitro study with shorter time releases of Az/ Vit C and IGF 1. Longer day release assessment with multiple growth factor release consideration in T-PRF as well as herbal extracts (TI/ NE) might have altered the results. Cell line studies, animal and huma clinical trials if performed after characterization with Scanning electron microscope (SEM) + Fourier Transmission Infrared Spectroscopy (FR-IR) can be helpful for proper establishment of T-PRF as SDDS of herbal extract.

CONCLUSION

Thus, within limitations present in-vitro study of T-PRF+TI and T-PRF+NE concludes that T-PRF can be used as SDDS and help in sustained release of herbal extract products such as Vit C, Az and IGF 1. Addition of herbal extracts didn't alter the release of growth factor. Further clinical trials with larger sample size should be performed to determine better conclusive outcomes.

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 (SRB/SDC/PhD/PERIO-2251/25/025).

Informed Consent

Verbal and written informed consent were obtained from healthy volunteers

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ORIGINAL RESEARCH

STUDY OF THE ORAL CAVITY AND DETERMINATION OF THE LEVEL OF PRO-INFLAMMATORY IL-2 AND ɤ-INF IN ORAL FLUID OF PATIENTS WITH VIRAL HEPATITIS C BEFORE AND AFTER COMPLEX TREATMENT

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ABSTRACT

Background: Interest in the study of combined pathology in recent times is explained by the accumulation of new facts, the emergence of new information on interorgan, intertissue and intercellular levels of interaction in the system of the whole organism. In this regard, the issue of the relationship between diseases of internal organs and organs of the oral cavity is relevant

The **aim** of the study was to examine the condition of the oral cavity and the level of proinflammatory IL-2 and ɤ-INF in the oral fluid of patients with viral hepatitis C before and after complex treatment.

Material and methods: The study involved 96 patients with HCV with lesions of the oral mucosa and periodontium, as well as 100 patients of the control group without HCV. All patients had their dental status and index assessment of the periodontal tissue condition studied. Cytokines IL-2, ɤ-INF were determined in the oral fluid. All indicators were studied before and after complex treatment and compared with each other.

Results: Objective examination of the oral cavity of patients with HCV revealed the presence of a generalized inflammatory process in the alveolar part of the gums, as well as damage to the OM ($p<0.001$). In HCV, pro-inflammatory IL-2 significantly increases, and ɤ-INF increases, but not statistically significantly ($p>0.113$). After complex treatment, statistically significant improvements are observed in the OM and alveolar part of the gum ($p<0.001$). Pro-inflammatory IL-2 decreased ($p<0.001$), and ɤ-INF decreased, but statistical significance is not observed ($p>0.121$).

Conclusion: Thus, a comparative study of lesions of the OM and alveolar part of the gingiva before and after the complex treatment for HCV revealed that the main pathognomonic symptoms reliably undergo dynamics towards improvement and are detected with a lower frequency. When comparing the results of interleukin levels in the OF before and after HCV treatment, a decrease in the level of proinflammatory IL-2 is noted, the level of ɤ-INF also decreases (but the difference in the data is insignificant). Further studies are needed to better understand the level of IL-c in patients with HCV and its relationship with damage to the oral mucosa and periodontium.

Key Words: Periodontal tissues, oral mucosa, index PI and SBI, cytokines, HCV.

INTRODUCTION

Interest in the study of combined pathology in recent times is explained by the accumulation of new facts, the emergence of new information on interorgan, intertissue and intercellular levels of interaction in the system of the whole organism. In this regard, the issue of the relationship between diseases of internal organs and organs of the oral cavity is relevant [1-3].

The study of combined lesions of the skin, internal organs, oral mucosa, their connection with general pathology is necessary for the doctor to make a correct diagnosis. There is a relationship between most pathological processes occurring between the oral mucosa, the red border of the lips and various organs and systems of the body, therefore, it is the lesions of the oral mucosa that are often the first signs of metabolic disorders, as well as various general somatic diseases [4].

According to modern concepts, the development and progression of inflammatory periodontal diseases are considered not only as a local inflammation of the periodontal tissues caused by the microflora of the "dental plaque", but also as a reaction of the body to a bacterial infection. Almost all researchers recognize that the imbalance between bacterial invasion and local resistance of the oral cavity is the main factor determining the development and course of periodontal lesions [5-7].

Many authors point to the high frequency and diverse clinical picture of oral lesions in diseases of the gastrointestinal tract, particularly viral liver diseases [8-11]. As a result of diseases of the digestive organs, the functional activity of the salivary glands, the composition and properties of saliva change, which leads to a violation of the dynamic balance of de- and remineralization processes, the development and active course of the carious process. An increase in the content of mucin, C-reactive protein, a decrease in the activity of lysozyme in oral fluid have been established, which indicates a violation of the non-specific resistance of the immune system in diseases of the digestive organs [12, 13].

The last decades of the 20th century and the beginning of the 21st century were marked by a sharp increase in the number of viral liver diseases, especially parenteral viral hepatitis, characterized by a high chronicity of the course, the development of multiple complications and mortality, which determined the medical and social importance of the problem of viral hepatitis [14,15]. The 63rd World Health Assembly (2010) recognized viral hepatitis B and C (HBV and HCV) as one of the leading health problems in the world [16, 17]. Chronic HCV affects 71 million people worldwide. It is most common in the WHO Eastern Mediterranean Region and the WHO European Region, where the prevalence of hepatitis C virus infection was estimated at 2.3% and 1.5%, respectively, in 2015. In other WHO regions, HCV prevalence rates range from 0.5% to 1%. In some countries, high rates of HCV may be found in specific population groups. For example, 23% of new HCV infections and 33% of HCV-related deaths are associated with injecting drug use. However, national HCV programmes do not always cover injecting drug users and prisoners [17].

The pathogenetic commonality of many general somatic processes and inflammatory diseases of the oral cavity is due to the development of mechanisms of cellular damage and modification of tissue structures with the acquisition of autoantigenic properties by them, which are common to the entire organism. The leading role in the occurrence of these changes is played by failures and dysfunctions of the cytokine regulation of immunobiological processes. The basis for the chronicity of any inflammatory process is the relationship between the periodontal conditions with proinflammatory properties and anti-inflammatory activity. In this regard, an in-depth study of non-specific detoxifying systems of the oral cavity in patients with various somatic diseases is of undoubted interest to dentists in order to clarify the genesis of damage to inflammatory diseases of the oral cavity and the general pathogenetic mechanism as a whole [18].

As is known, saliva or SS (unstimulated mixed saliva) is of great importance in ensuring mucosal immunity of the oral cavity; it contains a large number of substances with antimicrobial action: lysozyme, lactoferrin, immunoglobulins, antimicrobial peptides and other active substances [19]. The immunoglobulins contained in saliva, especially sIgA, prevent the adhesion of microorganisms, lysozyme destroys the bacterial walls, and lactoferrin deprives bacteria of iron [20]. In addition, saliva contains complement components and cytokines. Saliva testing is a valuable noninvasive method for assessing the general condition of the body and, in particular, the oral cavity. Saliva collection is convenient and simple, it is painless, the risk of infection of medical personnel is significantly lower than when working with blood, and the content of some substances in saliva (e.g. hormones, antibodies, drugs, etc.) reflects their concentration in the blood [21]. Modern technologies for studying proteins in biological environments make it possible to determine the levels of various immune indicators and their biological activity in saliva and other secretions containing the proteins being studied in minimal concentrations [22].

It is known from literary sources that, depending on the severity and duration of the process, the quantitative indicators of all subsystems of local immunity change. But the data on immune reactivity are varied and contradictory, most likely, this is due to different methods and techniques for assessing immune reactivity, the presence of systemic pathology, and the type of reactions [23]. As noted above, the development of inflammatory diseases is determined by the state of cytokine regulation. Most of both pro- and anti-inflammatory cytokines (such as IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, TNF-α, ɤ-IFN) are present not only in the peripheral blood, but also in saliva [24]. The sources of their production are both lymphocytes and macrophages embedded in the epithelium of the mucous membranes, and epithelial cells of the mucous membranes and the salivary glands themselves. Another source of cytokines in saliva may be their transudation from the blood serum. However, many researchers have noted that the content of cytokines in saliva does not correlate with their level in the blood, which indirectly indicates their local synthesis [25, 26].

Thus, the data from the study will help to develop tactics for conducting preventive methods of diagnostics and prevention of periodontal lesions, which is the basis for drawing up an individual treatment plan and determining the sequence of treatment and preventive measures, saving significant material costs on the part of patients and time costs on the part of specialists.

The **aim** of the study was to examine the condition of the oral cavity and the level of proinflammatory IL-2 and ɤ-INF in the oral fluid of patients with viral hepatitis C before and after complex treatment.

2.MATERILAS AND METHODS

The study included 196 patients before complex treatment, who were divided into 2 groups: Group I patients of the main group with HCV n= 96, Group II persons of the control group n=100. In addition to the main disease, patients with HCV had oral cavity lesions. The age of patients ranged from 37 to 63 years, hospitalized in the infectious diseases clinic of the Mikaelyan Institute of Surgery, Yerevan State Medical University (Yerevan, Armenia) during the period 2023-2025. The control group included 100 individuals without HCV with periodontal tissue and oral mucosa lesions who applied to the Stomatology Scientific and Educational Clinical Center No.1 of Yerevan State Medical University during the same period. Their age ranged from 21 to 57 years. The final diagnosis of HCV was established based on the detection of hepatitis C virus RNA in the blood using the PCR method.The patients underwent traditional clinical and laboratory examination methods, including general blood and urine tests, biochemical blood tests: determination of total bilirubin and fractions, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), ɤ-glutamyl transferase (GGT), total protein and fractions, coagulogram.

All patients underwent an assessment of their dental status according to previously developed criteria, which included an assessment of the condition of the marginal and alveolar parts of the gum, as well as various parts of the OM before and after complex treatment. An index assessment of the condition of the periodontal tissues was also performed according to the periodontal index (PI) according to Russell [27], and the gingival sulcus bleeding index (SBI) according to Miihlemann and Son was determined [28] before and after complex treatment. In accordance with the purpose of our work, all the above-mentioned examination methods, including determination of the level of cytokines in the gastrointestinal tract, were carried out in 50 patients of the main group after complex treatment. All patients with HCV received etiotropic treatment with antiviral drugs [17]. In addition to etiotropic treatment, patients in the main and control groups (n=50, respectively) received dental treatment (oral cavity sanitation) and a probiotic. The introduction of probiotics into clinical practice is one of the breakthroughs in medicine in the fight against infection. All patients received “Brefovil” sachets (manufacturer Sacura Italy, S.R.L.; active ingredients: Saccharomyces boulardi 8 mld UFC and zinc 10 mg). The drug was used locally in the form of rinses, 2 sachets for 10 days.

Study of cytokines in oral fluid (OF): Cytokines of the OF were tested among 45 patients with HCV and 30 patients without HCV, who agreed to pass this test. The test material was unstimulated mixed saliva – OF, obtained without stimulation and collected with a sterile syringe into sterile Eppendorf tubes. Samples were frozen and stored at –20 °C. The samples after were thawed at room temperature, centrifuged at 5000 rpm in the cold. Mucin was precipitated using 6 units of Lydase per 1.0 ml of OF by our patented method (Patent RA No. 3295 A dated at May 16, 2019) [30]. The concentrations of cytokines IL-2 and γ-INF was determined by the method of solid-phase enzyme-linked immunosorbent assay (ELISA) using the Vector-Best test systems (Vector-Best JSC, Novosibirsk, Russia) and was registrated on a Statfax 303 Plus photometer (Awareness Technology, Inc. Palm City, FL 34990, USA)

Statistical analysis: Descriptive analysis (Mean ± SD for continuous and frequencies/proportion for categorical variables) were calculated for all variables of interest. Differences between two groups were evaluated using “chi-square” or “Fisher’s exact” tests for categorical variables and “Wilcoxon signed rank test” for continuous variables. Spearmen correlation was performed for

determination of relationships between continuous variables. P-value was considered significant at <0.05 and <0.001 for highly significant results. Analyses were conducted using Excel 2013 and R software software and program Vassar Stats to calculate Odds Ratio (OR) and 95% Confidence Intervals (CI).

RESULTS

The study included 96 patients with HCV, 61 men (63.5%) and 35 women (36.5%). The control group involved 100 subjects without HCV with lesions of OM and periodontium: 62 men (62.0%) and 38 women (38.0%). The average age in HCV patients group was 50,05 ± 13,29, and in the control group 37.99 ± 16.66. Patient complaints and data from the clinical examination of the oral cavity were taken into account when examining the dental status, including: external examination of the lips and corners of the mouth, assessment of the state of various parts of the oral mucous and periodontium before complex treatment (Table 1). These results indicate that the **monolithic crowns** provide superior fracture strength performance compared to **bilayered crowns**, and the choice between **resin cement and glass ionomer cement** had no significant influence on the outcomes within each crown type (Table 5).

Table 1. Data from clinical examination of the oral mucosa and alveolar parts of the gums in patients with HCV and in control groups before complex treatment

Sign		Control group n=100		HCV n=96		p-value*
		absolute number	%	absolute number	%	
Disorders in the mucous membrane relief						
	Absent	97	97	10	10.4	
	Present	3	3	86	89.6	**<0.001**
Hemorrhages on the buccal mucosa and the hard palate		100	100	21	21.9	
	Absent	0	0	75	78.1	**<0.001**
	Present					
Telangiectasia on the buccal mucosa						
	Absent	100	100	31	32.3	
	Present	0	0	65	67.7	**<0.001**
Coated tongue	Absent	100	100	6	6.2	
	Present	0	0	90	93.8	**<0.001**
Hyperemia	Absent	99	99	77	80.2	
	Present	1	1	19	19.8	**<0.001**
Cyanosis	Absent	75	75	30	31.2	
	Present	25	25	66	68.8	**<0.001**
Bleeding	Absent	89	89	49	51	
	Present	11	11	47	49	**<0.001**

* p-value test result from the comparison between HCV and control group

According to the results of the examination of patients with HCV, disorders in the mucous membrane relief were detected in 89.6%, hemorrhages on the mucous membrane of the cheeks and hard palate were observed in 78.1%, telangiectasia was detected in 67.7% of patients, the presence of plaque on the surface of the tongue was detected in 93.8% of those examined with HCV (p<0.001). Hyperemia was observed in 19.8%, cyanosis - in 68.8%, bleeding gums were detected in 49% of those examined, which is statistically significant (p<0.001) compared to the control group. To assess the degree of periodontal damage in patients with HCV, the periodontal index (PI) according to Russel and the gingival sulcus bleeding index (SBI) according to Mühlemann and Son were determined before complex treatment (Table 2).

Table 2. Index assessment of the periodontal tissue condition in patients with HCV before complex treatment (mean± SD)

Sign	Control group	HCV	p-value*
PI, points	0.95±0.48*	4.51±0.64*	**<0.001**
SBI, points	1.68±1.66*	3.02±0.1*	**<0.001**

* p-value test result from the comparison between HCV and control group

The content of proinflammatory cytokines - IL2, ɤ-INF and anti-inflammatory cytokines - IL4 and IL10 in the oral fluid of patients with HCV before complex treatment was studied (Table 3).

Table 3. Oral fluid cytokines levels in the control group and in patients with HCV before complex treatment (mean ± SD)

Cytokines	Control group (n=30)	HCV (n=45)	Odds Ratio	95% CI	p value*
IL2	2.83±5.67	25.99±17.86	-23.17±2.86	[-28.89; -17.44]	**<0.001**
ɤ-INF	0.72±3.04	2.46 ±6.52	-1.74±1.12	[-3.98; 0.49]	>0.113

* p-value test result from the comparison between control and HCV groups

A comparative analysis of OF cytokine indices in HCV and in the control group revealed that the amount of pro-inflammatory IL-2 in HCV increases by 9.2 times ($p<0.001$), and the increase in ɤ-INF is statistically insignificant ($p>0.113$).Local treatment of the oral cavity was carried out against the background of etiotropic (antiviral) treatment of HCV according to the accepted protocol. In order to correct the disturbances of the microbiocenosis in the oral cavity, the probiotic "Brefovil" was used - 2 sachets per day for 10 days. Then an examination of the oral cavity was carried out, as well as determination of the final indicators of periodontal indices. The same level of IL in the OF was determined.The examination data of the OM and alveolar part of the gum after complex treatment in patients with HCV are presented in Table 4.

Table 4. Data from clinical examination of the oral mucosa and alveolar parts of the gums in patients with HCV before and after complex treatment

Sign		Before n=50		After n=50		p-value*
		absolute number	%	absolute number	%	
Disorders in the mucous membrane relief						
	Absent	8	16	27	54	
	Present	42	84	23	56	**<0.001**
Hemorrhages on the buccal mucosa and the hard palate						
	Absent					
	Present	10	20	28	56	
		40	80	22	44	**<0.001**
Telangiectasia on the buccal mucosa						
	Absent	16	32	32	64	
	Present	34	68	18	36	**<0.001**
Coated tongue	Absent	3	6	26	52	
	Present	47	94	24	48	**<0.001**
Hyperemia	Absent	40	80	44	88	
	Present	10	20	6	12	>0.18
Cyanosis	Absent	11	22	29	58	
	Present	39	78	21	42	**<0.001**
Bleeding	Absent	27	54	38	76	
	Present	23	46	12	24	**<0.001**

* p-value test result from the comparison between before and after complex treatment

In an objective study of the OM and periodontium in HCV, positive changes after complex treatment are obvious. Thus, after treatment, disorders in the mucous membrane relief - 1.5 times, hemorrhages on the mucous membrane of the cheeks and hard palate - 1.8 times and telangiectasia - 1.9 times less often than before treatment.

The difference between all these indicators before and after treatment was highly statistically significant ($p<0.001$). After treatment, the same patterns were observed in the study of the tongue. Thus, plaque on the surface of the tongue was detected almost 2 times less often ($p<0.001$). Of the symptoms indicating the presence of a generalized inflammatory process in the alveolar part of the gum, the following significantly changed after treatment: cyanosis ($p<0.001$) and bleeding ($p<0.02$). The detection of both symptoms decreased almost 2 times. The difference in the detection of hyperemia was statistically insignificant ($p>0.18$).

In patients with HCV, the periodontal index values differed statistically significantly before and after the complex treatment (Table 5).

Table 5. Index assessment of the periodontal tissue condition in patients with HCV before and after complex treatment (mean± SD)

Sign	Before treatment	After Treatment	p-value*
PI, points	4.25±0.78*	2.54±0.49*	**<0.001**
SBI, points	2.82±0.09*	1.74±0.31*	**<0.001**

* p-value test result from the comparison between before and after complex treatment

The PI and SBI index values decreased by more than 1.5 times with a high degree of reliability (p<0.001).

Studies of OF cytokines after the complex treatment for HCV showed that the content of pro-inflammatory IL-2 decreased almost 5 times (p<0.001), and the level of ɤ-INF decreased, however, the difference in data before and after treatment is statistically insignificant (p>0.121) (Table 6).

Table 6. Oral fluid cytokine levels in patients with HCV patients before and after complex treatment (mean ± SD)

Cytokines	Before treatment (n=45)	After treatment (n=45)	Odds Ratio	95% CI	p value*
IL2	25.99±17.86	4.98±11.76	21.01±3.19	[14.66; 27.36]	**<0.001**
ɤ-INF	2.46±6.52	1.58 ±6.89	0.89±1.41	[-1.93; 3.7]	>0.121

* p-value test result from the comparison between control and HCV groups

DISCUSSION

There is practically no pathology that would not affect the condition of the oral mucosa (OM). At the same time, the similarity of clinical manifestations in the oral cavity of diseases of different etiology and pathogenesis contributes to difficulties in making a final diagnosis [4, 31]. Most researchers consider the periodontium as an integral part of the whole organism and recognize the close pathogenetic connection between periodontal diseases and somatic pathology. Patients with periodontitis who have concomitant and background diseases require special attention, both in the diagnosis of combined pathology and in treatment and prevention [32]. Lesions of the OM and periodontium aggravate the course of the disease and serve as an important addition to the characteristics of the general clinical picture of HCV [33-35]. Viral liver lesions occupy an important place in the assessment of the state of dental health of patients [36]. At the same time, dentists and doctors of other specialties do not pay due attention to the state of the oral cavity in liver diseases [37]. The experience of a dentist working in infectious disease departments has shown that the effectiveness of diagnosis and treatment of OM lesions depends on the earliest possible examination of the admitted patient [38].

In a comparative study of the criteria we selected that characterize the state of various OM sections in HCV with the data of the control group, it was established that in the latter (except for isolated cases) OM lesions are practically not encountered. According to the results of our studies, pathognomonic symptoms of OM and periodontal lesions in HCV were reliably established [39,40].

Analyzing the frequency of occurrence of symptoms of lesions of the OM, it was found that in HVC, the leading positions with a high degree of reliability are occupied by two leading pathognomonic symptoms (disorders in the mucous membrane relief, the presence of plaque on the surface of the tongue) with fluctuations from 75.6% to 100% of cases. As for hemorrhages on the mucous membrane of the cheeks and hard palate, they are characteristic only of HCV. Foci of epithelial desquamation on the surface of the tongue and, especially, telangiectasia on the mucous membrane of the cheeks are more often detected in HCV [41].

The data on the condition of the marginal and alveolar parts of the gum in patients with HCV are of interest. As noted, only cyanosis is reliably more often established with HCV. It is appropriate to note that according to Fedeli U. et al. (2017), who studied the features of damage to the OM and periodontium in patients with chronic liver diseases of viral etiology, with chronic hepatitis and cirrhosis of the liver caused by the hepatitis B virus, more severe dystrophic and inflammatory changes are observed in the OM and periodontal tissues compared to those caused by the HCV [42]. Our data indicate that it is probably difficult to make a definitive conclusion regarding the

comparison of the severity of OM and periodontal damage in HCV, since some symptoms were detected significantly more often, while others had an insignificant statistical difference.

There are few works in the available literature that have studied and systematized the symptoms of damage to the OM and periodontium in HCV, especially in the early manifestations of the disease. The reliability of the frequency of occurrence of one or another symptom of the lesion has not been studied either. Some authors even point to the contradictory nature of the data indicating the connection between OM lesions and viral hepatitis [41]. In our opinion, these findings are related to the incorrectness of the research. Thus, Nagao Y. et al. (2014) studied the nature of OM lesions in patients suffering from chronic hepatitis (HBV - 20, HCV - 23 patients) and liver cirrhosis caused by HBV infection (15 patients) and HCV (16 patients). Presenting data on a small number of cases of OM damage using descriptive analysis, the authors came to the conclusion that there is no connection between chronic HBV and HCV, as well as the stage of the disease with the frequency and nature of OM damage [32]. Given the small number of patients and the lack of proper statistical analysis, one has to doubt the reliability of the authors' conclusions.

There are isolated works that describe individual, few signs in a small number of patients. No comparative analysis of data on the above signs in patients with viral hepatitis C has been conducted. In our opinion, this is important, since both patient management protocols and treatment regimens differ. It should be noted that the literature provides reviews on this problem, which describe in detail the epidemiological data and pathophysiological mechanisms of extrahepatic lesions in viral hepatitis [43-45]. However, there are few original studies in this area. We will try to discuss and compare those individual works that were found in the available literature.

Hepatitis C virus is a sialotropic virus. HCV-infected patients may often have features of Sjogren's syndrome such as sialoadenitis with mild or even absent clinical symptoms. The role of HCV in the pathogenesis of Sjogren's syndrome has not been fully studied. However, in 57% of cases of chronic liver diseases associated with HCV, histological changes in the salivary glands characteristic of Sjogren's syndrome were detected [46]. We did not set such a task for ourselves and therefore cannot conduct a comparative analysis of the obtained results.

The basis of the inflammatory process of any etiology is the launch of the cytokine cascade, which includes, on the one hand, proinflammatory cytokines, and on the other, anti-inflammatory mediators. The balance between the two opposing groups largely determines the nature of the course and outcome of the disease [26]. The main problem is the lack of accessible laboratory diagnostic methods that would clearly reflect the shift in the cytokine balance towards inflammatory or anti-inflammatory/immunosuppressive reactions. Given the multiplicity, as well as the synergism and pleiotropy of the cytokines involved in these reactions, it is clear that determining the concentration of any one of them in the blood will not adequately reflect the state of the entire cytokine balance. Perhaps only a one-time assessment of the level of several mediators (at least 2-3 of the opposing subgroups) may be more correct.

In connection with the above, we consider it appropriate to discuss some methodological aspects of our work in studying cytokines. The latter can come from the blood serum as a result of their transudation, but the content of cytokines in saliva does not correlate with their level in the blood, which indirectly indicates the prevalence of their local synthesis [25, 26]. Taking into account the recommendations of the literature, we studied the content of proinflammatory - interleukin 2 (IL-2), ɤ-interferon (ɤ-INF) in the oral fluid (OF), which is more accessible and non-invasive. As for age, it is clear that the average age of patients in the main group significantly differs (with HCV - 50.33 years) from the average age of the control group (26.93 years). This is explained by the fact that it was almost impossible to select patients in the control group in the age category from 40 to 50 years, who would not have any changes in the oral cavity. Patients in the main and control groups are not comparable in age, however, in our opinion, this is acceptable, since according to the literature, the composition of saliva undergoes changes with age only in older people [47,48]. According to WHO data, old age is from 60 to 75 years [49]. It was found that age in the groups we studied does not affect the level of OF cytokines [30,50].

There are a sufficient number of works in the literature devoted to the study of the content and ratio of pro- and anti-inflammatory cytokines in oral fluid in various pathologies [51-53]. However, it should be noted that the available data are very contradictory. This also applies to the data of the control groups, which creates certain difficulties in terms of a clear understanding of the normal cytokine levels in the oral fluid. There are isolated works in the literature devoted to the study of this issue in viral hepatitis C, but there are no works on the one-time study of proinflammatory (IL-2, ɤ-INF) in the same group of patients.

Given the high variability of the normal cytokine levels in the OF, we found it interesting to analyze the available literature data on the cytokines identified in our study. According to various authors, the IL-2 content in the OF in healthy individuals ranges from 0.1 ± 0.02 pg/ml to 10.0 (8.5; 28.5) [54, 55], ɤ-INF from 18.35 ± 0.47 to 23.8 ± 1.5 pg/ml [56]. Our data on IL-2 levels in the control

group coincide with the literature data, but there are discrepancies in ɣ-INF levels, which once again proves the high variability of the normal cytokine levels in the OF.

Polushina L.G. et al. (2017) determined the clinical and pathogenetic significance of some cytokines in periodontitis. According to their data, in patients with periodontitis, the concentration of IL-2 increased by 31.1% ($p<0.05$). In the same patients, a significant increase in the level of IL-4 by 26 times was observed ($p<0.001$). This gives grounds to assume that the cytokine balance in periodontitis is characterized by the predominance of Th2-produced factors, i.e. activation of anti-inflammatory immune-mediated mechanisms. The authors note that the obtained data correspond to the idea that the development of chronic periodontitis is accompanied by a violation of local immune mechanisms. This is manifested by an imbalance in cytokine production, primarily by the activation of IL-4 production. It can be assumed that this cytokine realizes its pathogenetic (immune-mediated tissue destruction) and protective (stimulation of antimicrobial immunity) effects in periodontitis. Their ratio most likely determines the rate of progression and development of complications of the disease [55].

There are a number of works in the literature devoted to the development of periodontitis in viral hepatitis [32, 53, 57-60]. However, there are practically no works on the study of cytokines in the GC in HCV. Therefore, the results of our studies partly coincide, and in some cases diverge from the literature data.

Thus, it has been proven that in patients with chronic hepatitis C, the course of periodontitis is characterized by more frequent and prolonged exacerbations with a shorter period of remission in comparison with the group of patients without background hepatitis. This is due to the development of periodontitis in patients with chronic hepatitis C against the background of endogenous intoxication, activation of lipid peroxidation processes and a decrease in enzymatic and non-enzymatic antioxidant potentials, caused primarily by chronic hepatitis. The author found that an increase in microbial contamination and activation of proinflammatory cytokines in periodontal pockets play a major role in the formation of a local inflammatory reaction in periodontitis against the background of chronic hepatitis C, and the degree of shifts in laboratory parameters significantly correlates with the severity of periodontitis [61,32].

A comparative study of OM lesions before and after treatment clearly shows that symptoms such as cracks in the corners of the mouth, disorders in the mucous membrane relief, and tongue plaque, which are common in viral hepatitis C, undergo almost identical reverse dynamics with a high degree of reliability ($p<0.001$) after complex treatment. Similarly, the indicators for the presence of hemorrhages on the mucous membrane of the cheeks and hard palate improve in viral hepatitis C. Desquamation of the epithelium on the tongue surface, on the contrary, is more often detected in HCV with reliable dynamics towards reverse development after complex treatment. As for telangiectasias, the latter is a pathognomonic symptom of HCV, undergoing reliable reverse dynamics after treatment.

When studying the dynamics of changes in the main criteria for damage to the marginal and alveolar part of the gum before and after treatment, it was found that with viral hepatitis C, only some symptoms with varying degrees of reliability undergo reverse dynamics.

We also studied the content of cytokines in oral fluid after treatment. When comparing the results for viral hepatitis C, the following pattern is observed: a decrease in the level of proinflammatory IL-2. When comparing the results of these indicators before and after treatment, the difference in data is highly reliable. The level of ɣ-INF also decreases, but the difference in data is unreliable.

The problem of liver diseases of viral etiology is still extremely relevant due to their wide distribution. The global coverage of territories and the high epidemic potential of this group of diseases maintain their social and economic significance. Pathologies caused by HCV are most often found in young, working-age individuals, leading to disability and fairly high mortality. Thus, the study of the state of the OM and periodontium in viral hepatitis C, the features of the cytokine profile of the RG and their comparative assessment before and after treatment is very relevant, in connection with which our study was conducted.

4.CONCLUSION

Thus, a comparative study of lesions of the OM and alveolar part of the gingiva before and after the complex treatment for HCV revealed that the main pathognomonic symptoms reliably undergo dynamics towards improvement and are detected with a lower frequency. When comparing the results of interleukin levels in the OF before and after HCV treatment, a decrease in the level of proinflammatory IL-2 is noted, the level of ɣ-INF also decreases (but the difference in the data is insignificant). Further studies are needed to better understand the level of IL-c in patients with HCV and its relationship with damage to the oral mucosa and periodontium.

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Consent for publication

Informed consent was obtained from every participant for documentation and examination.

Competing interests

The authors declare no competing interests.

Ethical approval

Ethical approval was granted by the Institutional Human Ethical Committee

Informed patient consent

All patients' clinical records were obtained with informed consent.

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ORIGINAL RESEARCH

THE EFFECT OF TOPICAL A-MANGOSTIN DERIVED FROM MANGOSTEEN PEEL (GARCINIA MANGOSTANA LINN) ON THE HEALING OF ACUTE FULL-THICKNESS WOUNDS IN RATS (RATTUS NORVEGICUS)

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ABSTRACT

Background: Wound healing is a complex biological process involving three main phases: inflammation, proliferation, and tissue remodeling. Full-thickness wounds, which extend through the entire skin layer down to the subcutaneous tissue, require effective therapeutic interventions to accelerate regeneration. Garcinia mangostana peel extract, particularly its active compound α-mangostin, has demonstrated antioxidant, anti-inflammatory, and antimicrobial properties. However, in vivo evidence regarding its topical effect on specific wound healing parameters remains limited.

Methods: This laboratory-based experimental study used a randomized post-test only control group design with 16 male Wistar rats (12 weeks, 180–200 g) divided into four groups: control day-5, control day-10, treatment day-5, and treatment day-10. A 2×2 cm full-thickness wound was created dorsally under ketamine anesthesia. Treatment groups received 1% α-mangostin topical gel formulated from >90% purified extract. Wound tissues were collected on days 5 and 10 for histopathological evaluation. Healing was assessed through epithelial thickness, angiogenesis, and collagen density using hematoxylin-eosin (HE) and Masson's Trichrome (MT) staining. Statistical analysis was performed using SPSS v27 (Shapiro-Wilk, independent t-test, and Mann-Whitney; $p < 0.05$ considered significant).

Results: On day 10, the treatment group showed significantly higher epithelial thickness ($p = 0.019$), angiogenesis ($p = 0.017$), and collagen density ($p = 0.004$) than controls. On day 5, angiogenesis ($p = 0.017$) and collagen density ($p = 0.027$) were also significantly improved, while epithelialization was not yet significant ($p = 0.098$).

Conclusion: Topical α-mangostin significantly enhances full-thickness wound healing by promoting early angiogenesis and collagen formation, followed by improved epithelial regeneration. It shows promise as a phytopharmaceutical candidate for wound therapy.

Keywords: α-mangostin; Garcinia mangostana; full-thickness wound; wound healing

INTRODUCTION

Full-thickness skin wounds, defined as injuries that extend through the entire dermis into the subcutaneous layer, remain a major clinical challenge. They are prone to infection, prolonged pain, delayed re-epithelialization and excessive scarring, all of which impair function and appearance and inflate healthcare costs.[1,2] Because wound repair is a tightly orchestrated sequence of inflammation, proliferation and remodeling, disruption of any phase can halt healing and lower quality of life for patients.[3,4]

Standard management in plastic and reconstructive surgery ranges from secondary intention healing and primary closure to skin grafting, commercial skin substitutes, negative-pressure wound therapy and topical growth factors.[5] Although these approaches shorten closure time, they are expensive, not universally available and may provoke immunological rejection or recurrent infection. These constraints have driven the search for complementary, safer and more affordable

wound therapies.[6]

Mangosteen peel (Garcinia mangostana Linn) has gained attention because it is rich in xanthones, with α-mangostin being the most abundant. In vitro, α-mangostin exhibits strong antioxidant, anti-inflammatory and broad-spectrum antimicrobial activities, notably against Staphylococcus aureus and Staphylococcus epidermidis.[7-9] Crude peel extracts down-regulate pro-inflammatory mediators such as COX-2, IL-6, IL-1β and nitric oxide, suggesting that they could temper the inflammatory phase of wound healing.[10] Because the compound is plant-derived, advocates argue that it offers good biocompatibility and lower systemic toxicity than many synthetic agents.[11]

The present study therefore aims to evaluate the effect of a well-characterized topical α-mangostin preparation, isolated from mangosteen peel, on the healing of acute full-thickness excisional wounds in rats (Rattus norvegicus). By monitoring macroscopic closure, histological parameters and inflammatory markers, we aimed to investigate whether α-mangostin accelerates and improves tissue repair compared with a standard ointment base. The findings support the compound's potential as a cost-effective adjunct for full-thickness wound management.

MATERIALS AND METHODS

Study design and animals

This laboratory experiment followed a randomized post-test only control group design. Sixteen male Wistar rats (Rattus norvegicus), 12 weeks old, 180–200 g, were housed six per polypropylene cage (30 × 40 × 15 cm) at 32 °C with free ventilation, PAR-G pellets (20 g day^{-1}) and water ad libitum. The minimum sample size of 16 animals was derived with the resource-equation method described in previous study.[12] After 7 days of acclimatization, rats were assigned by computer-generated random numbers to four groups (n = 4 each): control day-5 (C-5), control day-10 (C-10), treatment by α-mangostin day-5 (T-5) and treatment by α-mangostin day-10 (T-10). All procedures complied with the Guide for the Care and Use of Laboratory Animals and were approved by the Airlangga University Animal Care and Use Committee (No. 2.KEH.61.04.2025).

Topical α-mangostin formulation

Purified mangosteen-peel extract containing > 90 % α-mangostin (acquired from Andalas Sitawa Fitolab Co., Ltd., Indonesia) was dispersed in a carbopol hydrogel with propylene glycol, glycerin, triethanolamine, methyl paraben and sodium EDTA. α-Mangostin content was verified by high-performance liquid chromatography, and the gel was stored in opaque jars at 4 °C.

Surgical procedure and treatment protocol

Under intramuscular ketamine anesthesia (20 mg kg^{-1}), dorsal hair was clipped and disinfected with 10 % povidone-iodine followed by 1:30 Savlon. A single 2 × 2 cm full-thickness square wound was created with a sterile scalpel. Groups M-5 and T-10 received 0.1 g α-mangostin gel evenly spread over the wound; controls received no gel. All wounds were covered with a transparent semipermeable dressing and redressed twice daily at 08:00 and 18:00. On day 5 (C-5, T-5) or day 10 (C-10, T-10) rats were re-anaesthetized, the wound plus a 2 mm margin excised, and the animals euthanized by intraperitoneal pentobarbital (60–100 mg kg^{-1}). Specimens were fixed in 10 % neutral-buffered formalin.

Histological evaluation

Formalin-fixed tissues were embedded in paraffin, cut at 5 µm, and stained with both hematoxylin-eosin (HE) and Masson's trichrome (MT). A board-certified anatomical pathologist examined each slide with a Leica Flexacam i5 microscope at 400× magnification using Leica Enersight software v1.1.2. Five high-power fields per section were evaluated. Epithelial thickness and angiogenesis were graded 0–3 with the Gunawan scoring system, whereas collagen density was recorded as the raw percentage of MT-positive area in each field.[13]

Statistical analysis

Data were analyzed with SPSS v27. Normality was assessed by Shapiro–Wilk. Independent t-tests were applied to normally distributed data, meanwhile Mann–Whitney U-tests were used for abnormally distributed data. Significance was set at $p < 0.05$. Figures were prepared with GraphPad Prism v10.4.2.

RESULTS

All 16 rats completed the study without illness or mortality. Representative photographs of the 1 % α-mangostin hydrogel and surgical procedure are presented in Figure 1 and 2, respectively.

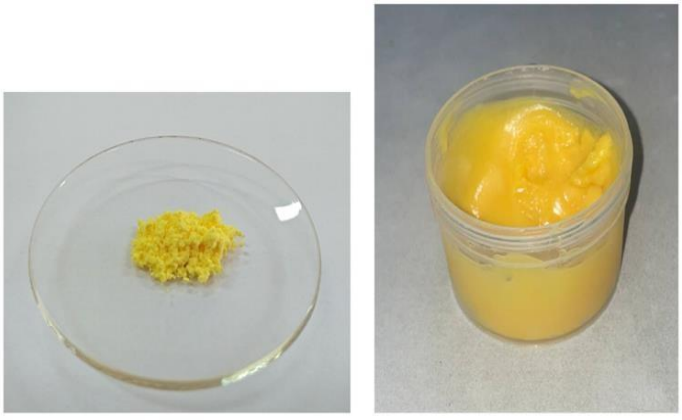


Figure 1. Purified α-mangostin powder (> 90 % purity) obtained from mangosteen peel (left) and the 1 % α-mangostin carbopol hydrogel formulated for topical application (right).

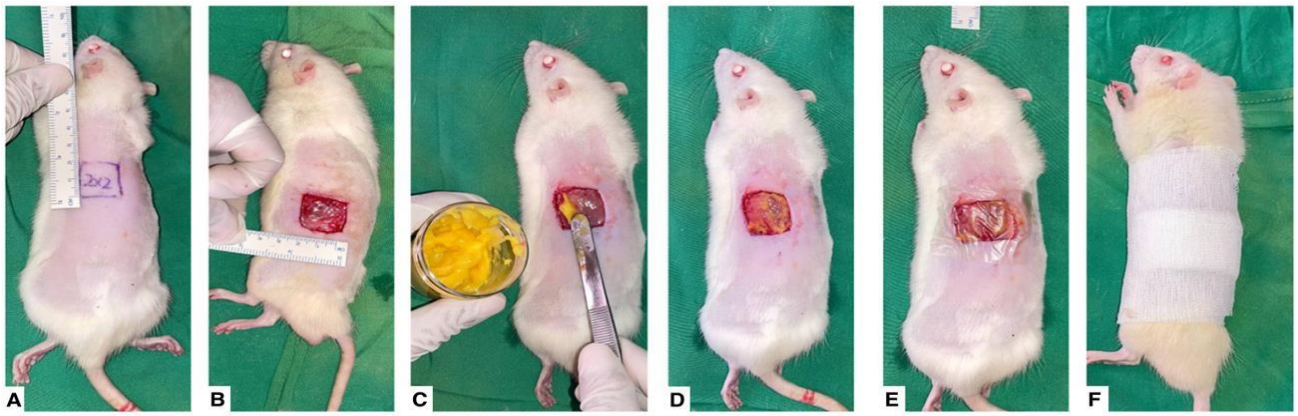


Figure 2. Step-by-step preparation of a 2 × 2 cm full-thickness dorsal wound in a Wistar rat: (a) shaving and marking the excision area, (b) creation of the wound, (c) topical application of 1 % α-mangostin hydrogel, (d) immediate wound appearance after treatment, (e) placement of a transparent semipermeable dressing, and (f) final bandaging.

Histological findings

Hematoxylin–eosin (HE) staining

By day 5, in the treated wounds a thin yet distinct epithelial layer was already present (green arrows). In the controls the epithelial rim was barely visible at the same magnification. Small new capillaries were common under the treated wounds (red arrows), while controls showed only a few such vessels and still contained many inflammatory cells and oedema. At day 10, re-epithelialization had occurred in both groups, but the epithelial layer was thicker and more orderly in the treated wounds. Capillaries were also more numerous and better formed than in the controls, where vessels were fewer and smaller (Figure 3).

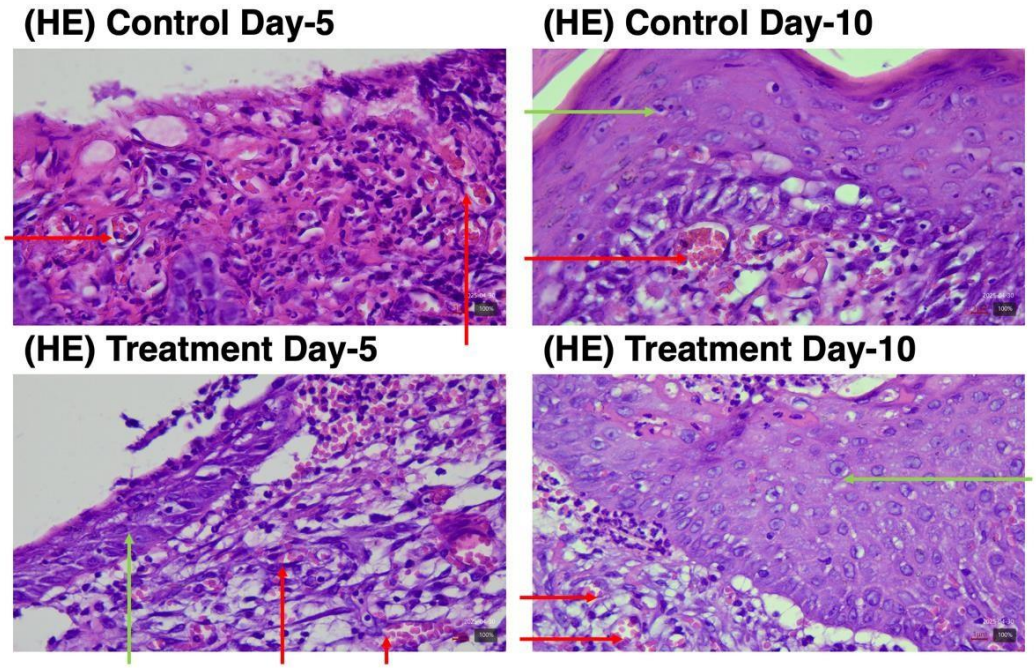


Figure 3. Representative hematoxylin–eosin (HE) micrographs of full-thickness wounds. Upper row: control groups on day 5 (left) and day 10 (right). Lower row: α-mangostin-treated groups on day 5 (left) and day 10 (right). Green arrows highlight the neo-epithelium; red arrows indicate newly formed blood vessels.

Masson's trichrome (MT) staining

By day 5, collagen in the control wounds appeared as faint, patchy blue areas with only a few thin bundles (white arrows). In the treatment group the blue signal was stronger and covered a wider area, showing that collagen deposition had begun earlier and was already denser than in controls. At day 10, treated wounds contained broad, compact, and mostly parallel collagen fibers that stained an intense blue. Control wounds still showed a light, uneven blue pattern with loosely organized fibers (Figure 4).

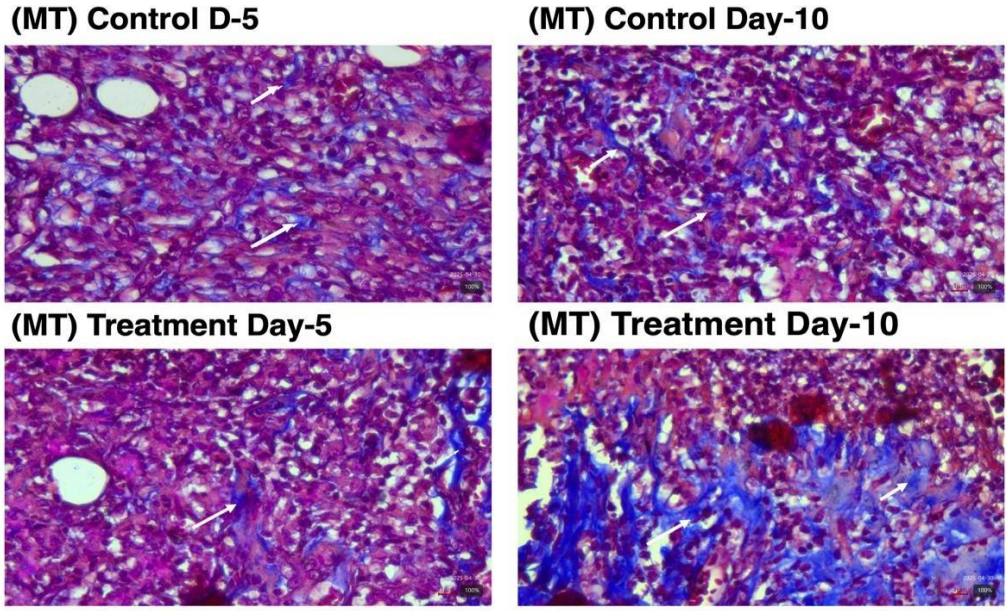


Figure 4. Masson's trichrome (MT) micrographs of full-thickness wounds. Upper row: controls on day 5 (left) and day 10 (right). Lower row: α-mangostin-treated wounds on day 5 (left) and day 10 (right). Blue staining marks collagen; white arrows highlight collagen bundles.

Statistical analysis

For epithelial thickness, the Mann–Whitney test detected a significant difference only between C-10 and T-10 ($p = 0.019$); C-5 vs T-5 and C-10 vs T-5 were non-significant ($p > 0.05$). An independent t-test showed that T-10 was also thicker than T-5 ($p = 0.002$). These findings indicate that the extract begins to increase epithelial thickness after day 5, with a clear effect by day 10. Angiogenesis followed a similar pattern. Mann–Whitney analysis showed higher vessel density in T-10 relative to C-10 ($p = 0.017$) and T-5 ($p = 0.036$), whereas C-5 vs C-10 was not significant ($p = 0.122$). The t-test confirmed that T-5 already exceeded C-5 ($p = 0.017$). Thus, the treatment enhances angiogenic activity from day 5 and this effect intensifies by day 10. Collagen density increased across all contrasts examined by t-test (all $p < 0.05$). Density rose over time in controls (C-5 vs C-10, $p = 0.017$) and was higher with treatment at each time-point (C-5 vs T-5, $p = 0.027$; C-10 vs T-10, $p = 0.004$). Within the treated group, T-10 also surpassed T-5 ($p = 0.021$). These results show that both wound age and topical α-mangostin additively promote collagen deposition (Table 1; Figure 5).

Table 1. Between-group analyses of each variables

Variable	Analysis between Groups		Group 1 Value	Group 2 Value	p-value
	Group 1	Group 2			
Epithelial thickness	Control Day-5†	Treatment Day-5†	0.2 (0.2)	0.3 (0.4)	0.098
	Control Day-10†	Treatment Day-5†	0.3 (0.2)	0.3 (0.4)	0.752
	Control Day-10†	Treatment Day-10†	0.3 (0.2)	0.1 (0.3)	0.019*
	Treatment Day-5‡	Treatment Day-10‡	0.35 ± 0.19	1.0 ± 0.16	0.002*
Angiogenesis	Control Day-5†	Treatment Day-10†	0.5 (0.5)	0.8 (0.2)	0.122
	Control Day-10†	Treatment Day-10†	0.8 (0.2)	1.3 (0.2)	0.017*
	Treatment Day-5†	Treatment Day-10†	1.0 (0.3)	1.3 (0.2)	0.036*
	Control Day-5‡	Treatment Day-5‡	0.5 ± 0.26	1.0 ± 0.16	0.017*
Collagen density	Control Day-5‡	Control Day-10‡	13.75 ± 8.54	28.75 ± 6.29	0.017*
	Control Day-5‡	Treatment Day-5‡	13.75 ± 8.54	32.50 ± 9.57	0.027*
	Control Day-10‡	Treatment Day-10‡	28.75 ± 6.29	52.50 ± 8.66	0.004*
	Treatment Day-5‡	Treatment Day-10‡	32.50 ± 9.57	52.50 ± 8.66	0.021*

*p-value significant at < 0.05, †Value displayed as median (IQR) and statistical analysis used Mann-Whitney test, ‡Value displayed as mean ± SD and statistical analysis used Independent t-test

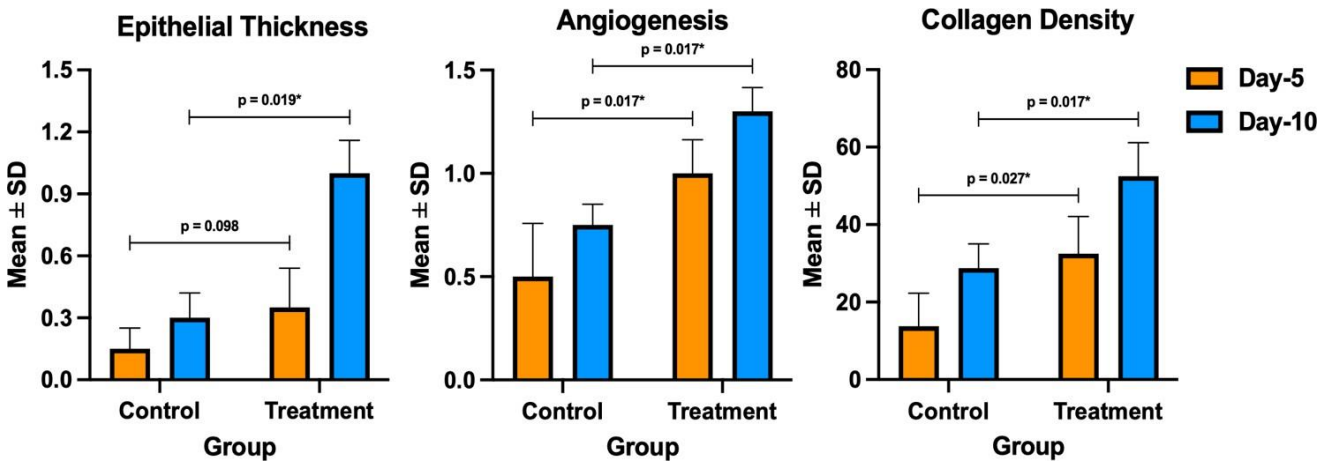


Figure 5. Comparative histograms illustrating epithelial thickness, angiogenesis score, and collagen density in control (C) and treatment (T) groups on day-5 (C-5, T-5) and day-10 (C-10, T-10).

DISCUSSION

This study demonstrates that topical application of α-mangostin (Garcinia mangostana Linn.) significantly enhances wound healing parameters in a full-thickness rat model, particularly by day 10. Three critical parameters, epithelialization, angiogenesis, and collagen density, all exhibited notable differences between the control and treatment groups by this time. On day 5, both angiogenesis and collagen density were significantly different, thus showcasing early effects of α-mangostin.[14]

The observed improvements align with the physiological phases of wound healing: inflammation, proliferation, and remodeling. Angiogenesis, crucially, supports the supply of oxygen and nutrients necessary for cellular activities such as fibroblast and keratinocyte migration and proliferation.[15] The early enhancement of angiogenesis on day 5 is considered a vital initiation that facilitates progression into re-epithelialization and extracellular matrix deposition. This modulation is likely attributed to the active compounds in mangosteen peel, especially α-mangostin, which is known for its potent antioxidant and anti-inflammatory effects.[16] The antioxidant properties help to mitigate local oxidative stress in the wound area, inhibit excessive inflammation, and enhance the release of angiogenic factors such as VEGF.[17]

Moreover, the significant increase in collagen density on both days 5 and 10 underscores collagen's essential role as a primary extracellular matrix component that provides tensile strength to the new tissue.[18] The data suggests that α-mangostin may stimulate fibroblast activation to produce collagen by modulating regenerative cytokines and growth factors. However, the specific molecular mechanisms need further investigation.

Interestingly, these findings differ slightly from a previous study which observed significant effects primarily on epithelialization using a 1% purified mangosteen extract.[7] The differences may stem from variations in topical formulation or the specific phyto-chemical composition in the non-purified extract. In addition, a supporting previous study corroborates that a 1% concentration of α-mangostin is optimal for promoting wound healing.[19]

The early significant effects on angiogenesis underscore its critical role as a trigger for the proliferative phase of healing. Enhanced vascularization ensures that healing cells receive an adequate supply of oxygen and growth factors necessary for effective recovery. Angiogenesis is essential for facilitating the subsequent processes of epithelialization and collagen remodelling, which are crucial for successful wound healing.[20]

In essence, this research reveals that α-mangostin accelerates wound healing through a gradual mechanism, starting with the enhancement of angiogenesis and collagen production in the early phase (day 5) and followed by accelerated epithelialization in the later phase (day 10). Its roles as an antioxidant, anti-inflammatory, and regenerative agent underscore its potential for developing safe and effective herbal-based topical therapies.

However, the study has limitations that must be considered. The relatively small sample size may affect statistical power and generalizability. Although key histological parameters were analyzed, specific molecular expressions such as VEGF or TGF-β, as well as enzymatic activities relevant to wound healing, were not evaluated. Furthermore, the study's focus was limited to day 10, omitting longer-term maturation and remodeling phases. Future studies should employ longitudinal designs and biomolecular approaches to further substantiate these findings.

CONCLUSION

In conclusion, the findings of this study clearly demonstrate the beneficial effects of topical α-mangostin (Garcinia mangostana Linn.) on wound healing in a full-thickness rat model. The treatment significantly enhanced epithelial thickness, angiogenesis, and collagen density, particularly noticeable by day 10. These results suggest that α-mangostin not only promotes the initial stages of healing, such as angiogenesis by day 5, but also facilitates later stages like epithelialization and collagen formation, contributing to the overall strength and integrity of the healed tissue. By highlighting the compound's multifaceted role in promoting wound healing, this study opens avenues for further research into its potential as a safe and effective herbal therapy. Future studies should aim to elucidate the specific molecular mechanisms involved and consider longer-term outcomes, further solidifying α-mangostin's role in wound care applications.

DECLARATIONS

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Competing and conflicting interests

The authors declare no competing interests or conflicts of interest related to this work.

Ethical approval

Ethical approval for this study was granted by the Airlangga University Animal Care and Use Committee (No. 2.KEH.61.04.2025), confirming adherence to ethical standards in the treatment of animal subjects.

Informed consent

Not applicable.

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