



ORIGINAL ARTICLE

EFFICIENCY OF HYALURONIC MOUTHWASH COMPARED TO CHLORHEXIDINE MOUTHWASH TO PREVENT PLAQUE INDUCED GINGIVITIS IN PATIENT WEARING FIXED ORTHODONTIC APPLIANCES

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Abstract

Background: Plaque-induced gingivitis is a prevalent periodontal condition affecting over 90% of individuals, with orthodontic appliances thus hindering effective oral hygiene.

Objectives: This study evaluated the efficacy of hyaluronic acid (HA) and chlorhexidine (CHX) mouthwashes in reducing gingival inflammation in patients with fixed orthodontic appliances.

Materials and Methods: A total of 45 participants were randomized into three groups: Group I used HA mouthwash, Group II used CHX mouthwash, and Group III brushed their teeth without a mouthwash. All participants underwent professional scaling and polishing before the study and followed standardized oral hygiene protocols. Saliva samples were collected at baseline (day 0), one month, and three months for measuring levels of pro-inflammatory cytokines (IL-1 β , TNF- α , and INF- γ). Gingival index (GI), plaque index (PI), and bleeding on probing (BOP) were assessed at each interval.

Results: At one month, significant reductions in GI, PI, and BOP were observed across all groups. Both groups of HA and CHX mouthwashes demonstrated greater reductions in clinical parameters compared to brushing alone. At three months, all groups showed continued significant improvements from baseline in all parameters except PI, which remained unchanged in the HA and brushing-only groups. Salivary cytokine analysis revealed variations correlating with clinical improvements, highlighting the anti-inflammatory benefits of both HA and CHX.

Conclusion: This study demonstrates that HA and CHX mouthwashes, alongside proper oral hygiene practices, effectively reduce gingival inflammation in orthodontic patients. These findings underscore the importance of integrating supportive periodontal therapies with rigorous oral hygiene regimens for maintaining oral health during orthodontic treatment.

Keywords: Gingivitis, Oral hygiene, Pro-inflammatory cytokines

INTRODUCTION

Plaque-induced gingivitis is a common periodontal disease that affects over 90% of people, irrespective of age, sex, or race¹. Orthodontic appliances often cause local soft tissue responses in the gingiva due to their proximity to the gingival sulcus and plaque buildup. These factors, along with difficulties in maintaining oral hygiene, complicate effective orthodontic care². The clinical effects observed following the insertion of orthodontic appliances into the oral cavity can lead to chronic infection, inflammatory hypoplasia, irreversible loss of attachment (permanent bone loss), and gingival recession³. The primary objective in preventing periodontal diseases is effective plaque control and the prevention of gingivitis⁴.

Effective plaque control can be achieved through various mechanical oral hygiene aids and numerous anti-plaque agents. Currently, mechanical methods for dental plaque removal are widely acknowledged as highly effective in controlling the progression of dental caries and periodontal diseases⁵. Mouth rinses and medical gels are typically considered supplementary to oral hygiene practices and are extensively used to deliver active agents to teeth and gingivae. These agents are frequently prescribed as adjuncts for the prevention or treatment of oral diseases because they inhibit bacterial colonization, growth, and metabolism, thereby disrupting the formation of mature biofilms at biochemical and ecological levels

^{6,7}. Chlorhexidine digluconate is recognized as a standard agent for plaque control; however, its prolonged use is often discouraged due to side effects such as tooth and tongue staining, altered taste sensation, and increased calculus formation⁸⁻¹⁰. In recent years, hyaluronic acid has garnered increasing attention due to promising results in treating inflammatory processes in various medical fields, including dentistry, dermatology, ophthalmology, and orthopedics^{11,12}.

Hyaluronic acid-containing mouthwash is an innovative product that incorporates a high molecular weight fraction of hyaluronic acid. Due to its non-toxicity, biocompatibility, and numerous biochemical and physicochemical properties, the topical application of hyaluronic acid-based biomaterials to periodontal pockets can be advantageous in enhancing healing and reducing periodontal inflammation¹³. This study aimed to evaluate the effectiveness of hyaluronic acid mouthwash (0.2%) and chlorhexidine mouthwash (0.12%) as preventive measures for plaque-induced gingivitis in patients with fixed orthodontic appliances.

MATERIALS AND METHODS

Ethical consideration and subject consent

The study proposal was approved by the scientific committee at the periodontal department and the ethical committee at the College of Dentistry, Hawler Medical University. Verbal and written informed consent were obtained from all participants before their participation in the study.

Study design and data collection

This clinical comparative study was conducted in the periodontal department at the College of Dentistry, Hawler Medical University, in Erbil city from March 2021 to March 2022. The study included 45 patients aged 18-25 years with plaque-induced gingivitis who were wearing full-mouth fixed orthodontic appliances. All subjects had good general health and possessed at least 24 natural teeth (excluding third molars), with a minimum of 30% bleeding on probing (BOP). Patients with systemic diseases, physical or mental disabilities, pregnant and lactating women, current smokers, those who had used antibiotics in the previous 6 months, or those who had received periodontal therapy in the previous 3 months were excluded from the study. Age, gender, educational status, and any history of systemic disease were assessed via interview on the day of the periodontal examination. Patients were randomly divided into three groups of 15 patients each:

Group I: Participants were instructed to use a mouthwash containing hyaluronic acid (Gengigel with 0.2% hyaluronic acid) in addition to brushing their teeth (after breakfast and before bed).

Group II: Participants were instructed to use a mouthwash containing Chlorhexidine Gluconate (KIN

Gingival Mouthwash with 0.12% Chlorhexidine digluconate), in addition to brushing their teeth (after breakfast and before bed).

Group III: Participants were instructed to brush their teeth only (after breakfast and before bed).

All participants received scaling and polishing by the same dentist using the same commercially available dentifrice (Colgate Total) and toothbrush. Participants in Group I and Group II were instructed to use their designated mouthwash nightly before bed, maintaining it in their mouth for 3 minutes without rinsing with water for one month.

Periodontal examination

A single experienced dentist conducted all periodontal examinations using standard dental equipment. The full mouth, excluding third molars, was assessed for plaque index (PI) and gingival index (GI) using Silness and Loe indices. Gingival sulcus depths (the distance from the marginal gingivae to the base of the gingival sulcus) and gingival recession (the distance from the cemento-enamel junction to the marginal gingivae) were measured with a manual periodontal probe and recorded to the nearest millimeter at four sites per tooth for all present teeth except third molars¹⁴. All periodontal examinations were performed three times: before starting therapy, after one month, and after three months.

Salivary samples collection

Saliva was collected from all participants at three different times: before starting therapy (day 0), and after one month (1-month) and three months (3-month). Saliva collection occurred prior to periodontal examination, with participants instructed to refrain from eating, chewing, and drinking for at least one hour beforehand. Unstimulated saliva samples were collected between 09:00 and 11:00 AM by asking participants to spit into a clean test tube until 5 ml of saliva was acquired. The samples were clarified by centrifugation at 10,000 g for 15 minutes. All samples were stored at -70 °C for subsequent analysis. Detection of salivary levels of IL-1 β , TNF- α , and INF- γ was performed in the laboratory following standard procedures and manufacturer's instructions^{15,16}.

Statistical analysis

Data analysis was conducted using IBM SPSS Statistics version 20. Descriptive statistics included mean and standard deviation for continuous variables and frequencies. A one-way ANOVA test compared group means.

RESULTS

The average male and females ages were 19.70 \pm 3.29 and 21.09 \pm 4.28 years with non-significant differences between both sexes (Table 1) in all groups. The mean values of plaque index (PI), bleeding on probing (BOP), and gingival index (GI) significantly decreased one month after therapy in the scaling and root planing (SRP) group,

demonstrating a notable difference compared to baseline measurements (0 day) prior to therapy (Table 2). After three months of SRP, the mean values of GI and BOP significantly increased compared to baseline, except for PI, which showed a non-significant decrease. However, no significant differences were observed between the 1- month and 3-month evaluations regarding all clinical periodontal parameters. concentrations were calculated using a standard curve. Osteocalcin levels were estimated using an electrochemiluminescence immunoassay (ECLIA) on the Roche Cobas e411 analyzer. The assay used biotinylated and ruthenium-labeled monoclonal antibodies in a sandwich format, and the chemiluminescent signal generated was proportional to osteocalcin concentration. All assays were performed and calibrated according to the manufacturers' instructions, and quality control

samples were used to ensure accuracy and precision.

Statistical analysis

All data were analyzed by using the Minitab program according to the ANOVA test. However, the mean when compromised by the ducun multiple range test under the P. value 0.05.

RESULTS

Effect of disease on the diagnostic parameters

Table 1 demonstrates statistically significant differences ($P < 0.05$) in several bone-related biochemical markers between diabetic patients and healthy controls. Specifically, serum levels of type I collagen, osteocalcin, and tartrate-resistant acid phosphatase (TRACP) were significantly elevated in patients compared to controls. These findings suggest enhanced bone turnover or metabolic alterations associated with diabetes mellitus.

Table1. The mean ages and standard deviation of male and female.

	Sex	N	Mean age \pm SD	Std. Error		p-value
Scaling	M	9	19.67 \pm 2.12	0.707		0.172
	F	6	22.67 \pm 5.75	2.348		
CHX ^a	M	8	18.25 \pm 0.46	0.164		0.065
	F	7	21.14 \pm 4.06	1.534		
HA ^b	M	6	21.67 \pm 5.68	2.319		0.490
	F	9	20.00 \pm 3.46	1.155		
Average total	M	23	19.70 \pm 3.30	0.687		0.226
	F	22	21.09 \pm 4.29	0.914		

^aChlorhexidine digluconate, ^b hyaluronic acid.

In relation to pro-inflammatory cytokines, the mean salivary levels of IL1- β , TNF- α , and INF- γ exhibited a significant increase after one month of therapy (Table 2). Subsequently, these levels showed a slight decrease after three months, with significant differences from baseline levels observed for IL1- β and INF- γ only. However, no significant differences were identified between the measurements taken at one and three months for TNF- α and INF- γ .

Table 2. The mean values of clinical and immunological parameters after 1 and 3 months of scaling and root planning.

			N	Mean \pm S.D.	t-test	d.f.	p-value	Sig.
Scaling	PI	0 day	15	1.77 \pm 0.46	4.49	14	0.001	HS
		1 month	15	1.54 \pm 0.38				
		0 day	15	1.77 \pm 0.46	0.81	14	0.434	NS
		3 months	15	1.67 \pm 0.58				
		1 vs 3						
	BOP	0 day	15	70.13 \pm 13.13	9.28	14	0.000	HS
		1 month	15	37.13 \pm 14.06				
		0 day	15	70.13 \pm 13.13	7.08	14	0.000	HS
		3 months	15	41.33 \pm 14.40				
		1 vs 3						
	GI	0 day	15	1.73 \pm 0.14	7.62	14	0.000	HS
		1 month	15	1.37 \pm 0.14				
		0 day	15	1.73 \pm 0.14	6.09	14	0.000	HS
		3 months	15	1.45 \pm 0.21				
		1 vs 3						
	TNF- α	0 day	15	14.34 \pm 2.65	-2.39	14	0.031	S

	IL-1 β	0 day	15	22.16 \pm 4.71	-2.49	14	0.026	S
		1 month	15	27.58 \pm 6.43				
		0 day	15	22.16 \pm 4.71	-5.41	14	0.000	HS
		3 months	15	33.64 \pm 5.29				
		1 vs 3			-3.07	14	0.008	HS
	INF- γ	0 day	15	27.78 \pm 5.43	-2.71	14	0.017	S
		1 month	15	34.97 \pm 6.45				
		0 day	15	27.78 \pm 5.43	-3.06	14	0.008	HS
		3 months	15	31.56 \pm 5.83				
		1 vs 3			1.36	14	0.197	NS

In the CHX group, there was a notable reduction in the mean values of PI, BOP, and GI after 1 month. However, after 3 months, the mean values of PI, GI, and BOP increased significantly compared to the baseline, with no significant differences observed between the 1 and 3-month evaluations. Similarly, the mean salivary levels of TNF- α and INF- γ increased significantly after 1 month of therapy, except for IL-1 β , which did not show a significant change. After 3 months, the mean values of pro-inflammatory cytokines decreased significantly when compared to the 1-month values for TNF- α and IL-1 β .

Table 3. Clinical and immunological parameters after 1 and 3 months of therapy by Chlorhexidine.

			N	Mean	t-test	d.f.	p-value	Sig.
CHX	PI	0 day	15	2.05 \pm 0.57	5.04	14	0.000	HS
		1 month	15	1.46 \pm 0.18				
		0 day	15	2.05 \pm 0.57	5.52	14	0.000	HS
		3 months	15	1.53 \pm 0.23				
		1 vs 3			-1.45	14	0.170	NS
	BOP	0 day	15	61.73 \pm 14.97	5.52	14	0.000	HS
		1 month	15	34.40 \pm 9.05				
		0 day	15	61.73 \pm 14.97	5.58	14	0.000	HS
		3 months	15	39.40 \pm 13.59				
		1 vs 3			-1.54	14	0.145	NS
	GI	0 day	15	1.67 \pm 0.25	4.96	14	0.000	HS
		1 month	15	1.34 \pm 0.09				
		0 day	15	1.67 \pm 0.25	4.91	14	0.000	HS
		3 months	15	1.39 \pm 0.14				
		1 vs 3			-1.65	14	0.121	NS
	TNF- α	0 day	15	13.42 \pm 3.06	-4.08	14	0.001	HS
		1 month	15	20.45 \pm 6.00				
		0 day	15	13.42 \pm 3.06	8.06	14	0.000	HS
		3 months	15	8.32 \pm 1.18				
		1 vs 3			7.66	14	0.000	HS
	IL-1 β	0 day	15	31.14 \pm 4.85	-1.15	14	0.269	NS
		1 month	15	33.46 \pm 6.92				
		0 day	15	31.14 \pm 4.85	8.69	14	0.000	HS
		3 months	15	17.77 \pm 4.68				
		1 vs 3			9.52	14	0.000	HS
	INF- γ	0 day	15	29.88 \pm 6.24	-5.69	14	0.000	HS
		1 month	15	37.85 \pm 7.90				
		0 day	15	29.88 \pm 6.24	-1.57	14	0.138	NS
		3 months	15	34.08 \pm 8.20				
		1 vs 3			1.16	14	0.266	NS

For the subjects who received HA treatment (Table 4), the mean values of PI, BOP, and GI significantly decreased after

1 month. After 3 months, the mean value of GI increased significantly compared to the baseline (day 0) and 1 month. The mean value of PI slightly increased after 3 months, with no significant differences compared to the baseline and 1 month. For BOP, the mean values significantly decreased after 3 months of therapy, showing significant differences from both the baseline and 1 month. The average salivary levels of TNF- α , INF- γ and IL-1 β increased insignificantly after 1 month of HA therapy and decreased slightly after 3 months. The comparison between each of two groups and between the three studied groups in regard to PI, GI and BOP showed that there was no significant difference between them (Data not shown).

Table 4. Clinical and immunological parameters after 1 and 3 months of therapy by Hyaluronic acid.

			N	Mean \pm SD	t-test	d.f.	P-Value	Sig.
HA	PI	0 day	15	1.77 \pm 0.26	3.37	14	0.005	HS
		1 month	15	1.54 \pm 0.21				
		0 day	15	1.77 \pm 0.26	0.62	14	0.543	NS
		3 months	15	1.71 \pm 0.30				
		1 vs 3			-1.86	14	0.083	NS
	Bop	0 day	15	63.13 \pm 9.54	18.54	14	0.000	HS
		1 month	15	28.00 \pm 9.30				
		0 day	15	63.13 \pm 9.54	9.291	14	0.000	HS
		3 months	15	45.47 \pm 8.28				
		1 vs 3			-10.30	14	0.000	HS
	GI	0 day	15	1.65 \pm 0.13	9.80	14	0.000	HS
		1 month	15	1.28 \pm 0.13				
		0 day	15	1.65 \pm 0.13	6.56	14	0.000	HS
		3 months	15	1.44 \pm 0.16				
		1 vs 3			-4.82	14	0.000	HS
	TNF- α	0 day	15	15.90 \pm 4.05	-1.41	14	0.180	NS
		1 month	15	18.58 \pm 5.60				
		0 day	15	15.90 \pm 4.05	0.60	14	0.559	NS
		3 months	15	14.97 \pm 6.01				
		1 vs 3			2.38	14	0.032	S
	IL-1 β	0 day	15	31.76 \pm 8.13	-0.81	14	0.429	NS
		1 month	15	34.41 \pm 6.54				
		0 day	15	31.76 \pm 8.13	5.59	14	0.000	HS
		3 months	15	16.01 \pm 3.84				
		1 vs 3			9.11	14	0.000	HS
	INF- γ	0 day	15	26.77 \pm 6.52	-2.02	14	0.063	NS
		1 month	15	31.40 \pm 9.23				
		0 day	15	26.77 \pm 6.52	-1.17	14	0.260	NS
		3 months	15	30.02 \pm 8.90				
		1 vs 3			-0.67	14	0.515	NS

The comparisons of pro-inflammatory cytokine level changes among the groups are summarized in Table 5. For TNF- α levels, no significant differences were observed between the treatment groups (HA and CHX) after 1 month. However, by the 3-month mark, significant differences were noted between the CHX group and the other two groups. Regarding IL-1 β levels, no significant differences were observed between CHX and HA. After 1-month, significant differences emerged between the scaling and HA groups, as well as between CHX and the other two groups.

Table 5. Levels of pro-inflammatory cytokines in the three groups.

		N	Mean \pm SD	F-test	P-Value	Sig.
TNF-0	Scaling	15	14.34 \pm 2.65	2.16	0.128	NS
	CHX	15	13.42 \pm 3.06			
	HA	15	15.90 \pm 4.05			
TNF-1	Scaling	15	17.99 \pm 4.77	0.82	0.445	NS
	CHX	15	20.45 \pm 6.00			
	HA	15	18.58 \pm 5.60			
TNF-3	Scaling	15	16.17 \pm 2.92	17.45	0.000	HS
	CHX	15	8.32 \pm 1.18			
	HA	15	14.97 \pm 6.01			
IL1-0	Scaling	15	22.16 \pm 4.71	11.60	0.000	HS
	CHX	15	31.14 \pm 4.85			
	HA	15	31.76 \pm 8.13			
IL1-1	Scaling	15	27.58 \pm 6.43	4.66	0.015	S
	CHX	15	33.46 \pm 6.92			
	HA	15	34.41 \pm 6.54			
IL1-3	Scaling	15	33.64 \pm 5.29	65.68	0.000	HS
	CHX	15	17.77 \pm 4.68			
	HA	15	16.01 \pm 3.84			
INF-0	Scaling	15	27.78 \pm 5.43	1.02	0.370	NS
	CHX	15	29.88 \pm 6.24			
	HA	15	26.77 \pm 6.52			
INF-1	Scaling	15	34.97 \pm 6.45	3.85	0.029	S
	CHX	15	37.85 \pm 7.90			
	HA	15	30.02 \pm 8.90			
INF-3	Scaling	15	31.56 \pm 5.83	0.54	0.584	NS
	CHX	15	34.08 \pm 8.20			
	HA	15	31.40 \pm 9.23			

At 3 months, no significant differences were found between the HA and CHX groups; however, significant differences were noted between the scaling group and both the HA and CHX groups, as well as among all three groups. For INF levels, no significant differences were observed between any two groups or among all three groups at baseline and at 3 months. The only exception occurred after 1 month, when significant differences were found between CHX and HA, as well as between the scaling group and the other two groups.

DISCUSSION

Fixed orthodontic periodontal therapy is a common treatment for different type of mal-occlusion, however fixed orthodontic appliances enhance in dental biofilm accumulation and make it difficult to be removed by routine oral hygiene procedures which intern result in periodontal inflammation¹⁶. Chlorohexidine gluconate is a gold stander for treatment and prevention of periodontal diseases caused by dental biofilm accumulation, but long-term usage of Chlorohexidine gluconate can result in many drawbacks including dental staining, taste alteration¹⁰.

Hyaluronic acid (HA) is essential for the function of extracellular matrices in periodontal tissues (17). HA plays an important role in the mechanisms underlying inflammation and wound healing. It exhibits potential in the regulation of periodontal tissue regeneration and in the treatment of periodontal disease¹³. Moreover,

HA has antibacterial, anti-inflammatory, osteo-inductive activities, and it enhances wound healing. When introduced as a local chemotherapeutic agent, it exhibited numerous clinical therapeutic properties and showed anti-inflammatory and antibacterial properties for the treatment of periodontal disease¹⁸.

The objective of this study was to evaluate the clinical and immunological parameters after scaling and polishing alone compared to those using chlorhexidine or hyaluronic acid after scaling in subjects wearing fixed orthodontic appliances. This study involved 45 participants wearing fixed orthodontic appliances in which 22 were female and 23 were male. This study found that all of GI, PI and BOP were reduced significantly after one month of study in all three groups (scaling and polishing alone, scaling and polishing with using chlorhexidine or HA mouth washes for 1 month once at night before bed. All three periodontal therapies plus oral hygiene instruction and performance by the patient are

effective in decreasing periodontal inflammation in patients wearing fixed orthodontic appliances.

After 3 months, there were statistically significant decreases in all previous clinical parameters comparing to base line in all three groups except for the plaque index in two groups, the HA group and the group that did not use any mouth wash, which indicates that maintaining good oral hygiene can result in maintaining oral health provided by periodontal therapy (19). Two recent studies observed similar outcomes, indicating that after a 3-month follow-up, CXH remains effective in reducing GI and PI when used alongside proper tooth brushing (20,21). Furthermore, other reports documented the effectiveness of HA gel combined with scaling and polishing in reducing plaque induced gingivitis than scaling and polishing alone²²⁻²⁴. May be due to that, HA reduces inflammation by modulating cytokines such as IL-1 β and TNF- α , which are elevated in gingivitis. HA promotes fibroblast proliferation and extracellular matrix formation, aiding gingival healing. It has bacteriostatic properties that can help reduce plaque accumulation. In addition to forming a protective layer over gingival tissues, reducing irritation from plaque and bacteria. Other researchers also suggested that other mouth washes and therapeutic modalities are effective as much as Chlorohexidine mouth washes in reducing gingivitis in patients wearing fixed orthodontic appliances^{23,25-27}.

The mean salivary level of IL-1 β significantly increased as compared to base line in scaling group but increase slightly in other 2 groups. After 3 months of therapy, the salivary level of IL1 β was significantly decreased in both studies groups but still significantly increased in scaling group. This result is consistent with the outcome of the other researchers²⁸. Comparing the salivary level of IL-1 β among the groups, after one month of intervention, significant difference existed between scaling and HA groups and between CHX and the other two groups with the scaling group had lowest level. After 3 months, no significant difference existed between HA and CHX groups with significant differences existed between scaling and the two studied HA and CHX groups with the scaling group had the highest level. While the mean salivary level of TNF- α was increased significantly after one month as compared to base line in scaling group and in the group using chlorohexidine mouth wash but this increase in the salivary level were not significant in a group using hyaluronic mouth wash. After 3 months of introversion, the mean salivary level of TNF- α was decreased significantly in the group using chlorohexidine mouth wash but this reduction was not significant in other 2 groups.

However, comparing the levels among the groups there were no statistical differences among the three groups after 1 month but after 3 months the level was significantly lower in the group using chlorohexidine mouth wash compared to the other 2 groups. The interpretation for this changes by short term increase in IL-1 β ; that, SP alone triggers a significant rise in IL-1 β due to mechanical irritation and inflammation; while, HA gel + SP results in only a slight increase, likely because HA's anti-inflammatory and healing properties counteract excessive cytokine release. The long term response in both study groups (SP + HA or other adjunctive therapy) showed IL-1 β levels decrease significantly, indicating reduced inflammation and improved periodontal healing. In contrast, the SP-only group still shows elevated IL-1 β levels, suggesting persistent low-grade inflammation. The HA gel as an adjunct to SP helps in modulating the inflammatory response, promoting faster and more effective healing. Patients undergoing SP alone may experience prolonged inflammation, which could increase susceptibility to gingival irritation and disease recurrence. IFN- γ is involved in bone remodeling during orthodontic tooth movement, which strongly suppresses osteoclastogenesis and has a role in periodontal inflammation²⁹ and its level significantly increased in periodontitis patient (30). In the current research, after 1 month of therapy salivary level of INF- γ was significantly increased in the scaling group and the group using chlorohexidine mouth wash, but this elevation was not significant in the group using hyaluronic acid mouth wash. After 3 months of therapy salivary level of INF- γ was significantly decreased in the group using chlorhexidine mouth wash but this reduction was not significant in other 2 groups. Comparing the three groups, after 1 month of therapy there were significant differences among them with group using hyaluronic acid had the lowest level of salivary INF- γ and CHX group had the highest level, that may be attributed to that HA mouthwash has the least impact on IFN- γ levels, suggesting a more balanced immune response with reduced inflammation, while; CHX leads to an initial spike in IFN- γ (possibly due to its cytotoxic effects), but over time it significantly reduces inflammation. SP alone induces prolonged inflammation, as evidenced by increased IFN- γ without a significant reduction after 3 months. This is agreed with other reports^{31,32}.

CONCLUSION

All the clinical parameters (GI, PI, and BOP) significantly decreased in all three groups (scaling and polishing alone, scaling and polishing with chlorhexidine mouthwash, and scaling and polishing with HA mouthwash) after 1 month. Each therapy, combined with oral hygiene instruction and patient compliance, was effective in reducing periodontal inflammation in patients with fixed orthodontic appliances. After 3 months, significant reductions in all clinical parameters were observed across all groups

compared to baseline, except for the plaque index in the HA mouthwash group and the group without any mouthwash. This highlights that maintaining good oral hygiene, supported by periodontal therapy, is crucial for sustaining oral health.

DECLARATIONS

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Competing Interests

The authors have no competing interests to declare.

Ethical Approval

The study was approved by the appropriate ethics committee and conducted according to relevant guidelines and regulations.

Informed Consent

Not applicable

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