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A Randomized Clinical Trial on the Clinical and Immunological Efficacy of a Mouth Rinse Containing Hydrogen Peroxide and Hyaluronic Acid in Patients with Gingivitis.

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Abstract

OBJECTIVE: The present study aimed to investigate the impact of HA/H_2O_2 (Hyaluronic Acid/Hydrogen Peroxide) mouthwash on pro-inflammatory cytokines, specifically IL-1β and IL-6, in individuals diagnosed with gingivitis. Furthermore, a comparison was made between the effects of HA/H_2O_2 and those of CHX (Chlorhexidine) and placebo.

MATERIALS AND METHODS: This clinical trial was randomized, double-blinded, and parallel. Fifty-four students with biofilm-induced gingivitis (18 to 23 years old) were randomly assigned to three mouthwash groups: HA/H_2O_2, CHX, and placebo. Unidentified bottles were delivered to participants. For 14 days, 10 millilitres of HA/H_2O_2 mouthwash formulation, CHX (0.12%), and placebo were used twice a day. All subjects were assessed twice, once at baseline and once after 14 days. Before and after each participant's mouthwash usage, bleeding on probing was measured as a clinical parameter for inflammation, Salivary IL-1β and IL-6 were also assessed by ELISA. Changes in salivary interleukins and bleeding between baseline and after treatment were determined and compared by the *t* test with an alpha of 0.05.

RESULTS: There was a statistically significant decrease in salivary interleukin-1β and IL-6 in all three groups. However, intergroup comparisons at second visits showed no significant difference between HA/H_2O_2 and CHX groups (P>0.05) for the interleukins tested. Furthermore, all treatments considerably reduced bleeding on probing, although mouthwash with hyaluronic acid (HA) and hydrogen peroxide (H_2O_2) had a greater impact than the other two treatments.

CONCLUSION: Mouthwash containing HA/H_2O_2 demonstrated an anti-inflammatory effect, suggesting they are immunomodulatory agents. These findings may be beneficial and encouraging for the use of HA/H_2O_2 in the treatment of gingivitis; thus, HA/H_2O_2 rinse has the potential to serve as an appropriate replacement for chlorhexidine (CHX).

KEYWORDS: Hyaluronic acid; Interleukin-1β; Hydrogen peroxide; Gingivitis; Saliva

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Introduction

Gingivitis is an inflammatory reaction caused by the accumulation of bacteria-in the plaque around the margin of the gingival tissue [1,2].The prevalence of biofilm-induced gingivitis is widespread among human populations and represents a primary periodontal ailment. If left untreated during its initial stages, this condition has the capacity to escalate into more severe forms of periodontal disease [3]. Cytokines are a category of soluble proteins with small molecular weight that are synthesized in response to an antigenic stimulus. They serve as chemical mediators that regulate the immune system that is innate as well as adaptive [4]. Interleukin-1 beta (IL-1) is a potent

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pro-inflammatory mediator that binds to IL-1 receptor and is essential for host defense responses to infection and injury [5]. It has been reported that IL-1 in the inflammatory site is responsible for increased local blood flow, leucocyte recruitment, and neutrophil infiltration [6]. Interleukin-6 (IL-6) is a cytokine that exhibits diverse physiological effects, such as the differentiation and activation of macrophages and T cells, the growth and differentiation of B cells, the stimulation of haematopoiesis, the differentiation of osteoclasts, and the resorption of bone [7].

The mouthwash containing chlorhexidine (CHX) is considered the preferred option for complementing oral hygiene due to its exceptional effectiveness in preventing the development of dental biofilm [8,9]. Nevertheless, the utilisation of this substance may result in various adverse effects, such as discoloration, modified gustatory perception, and infrequently enlargement of the parotid gland [10].The development of alternative mouthwashes with comparable efficacy, but without these adverse effects is desirable. The present study aimed to examine the efficacy of a mouth rinse solution comprising of hydrogen peroxide (H_2O_2) and hyaluronic acid (HA). H_2O_2 possesses oxygenating properties and exhibits antimicrobial activity by releasing oxygen. It has been demonstrated to be efficacious against both Gram-positive and Gram-negative bacterial strains [11,12]. HA is a linear polysaccharide that occurs naturally and serves as a crucial constituent of the extracellular matrix in various human tissues [13]. Additionally, HA is present in the periodontal ligament, as well as gingivae, while the cementum and alveolar bone contain minimal amounts of this substance [14]. HA is involved in phagocytosis, cell migration, adhesion, and wound healing due to its anti-inflammatory and anti-bacterial characteristics [15]. The objective of this investigation was to assess the impact of a rinse containing (HA+ H_2O_2) on pro-inflammatory cytokines, specifically IL-1B and IL-6, among individuals diagnosed with gingivitis, and to juxtapose these results with those obtained from CHX and a placebo solution.

Material and Methods

Experimental Design

This parallel three-arm double-blinded clinical trial was conducted between March and April 2023 at University of Baghdad's Faculty of Dentistry. The Helsinki Declaration and CONSORT 2010 Statement for multi-arm studies were followed. Volunteers visited the hospital's dentistry clinics at the start and end of the 14-day study. The Research Ethics Committee of the University of Baghdad, Faculty of Dentistry approved the study (Project No. 748622, December 28-2022). In 2023, “Clinicaltrials.gov” registered this clinical trial under protocol number “NCT05787600”.

Study Subjects

The trial's sample comprised of undergraduate students who were receiving treatment at the dental clinics of University of Baghdad's Faculty of Dentistry. A notice was displayed in the reception area of the clinics, soliciting the participation of students as volunteers in the research investigation. Individuals who satisfied the predetermined criteria for inclusion were extended an invitation to partake in the present investigation.

The study's inclusion criteria encompassed individuals who were in good systemic health, did not smoke, possessed over 20 natural teeth, and had been diagnosed with biofilm-induced generalised gingivitis. This diagnosis was characterised by the presence of over 30% bleeding sites with no PPD greater than 3 mm, an intact periodontium, and no loss of periodontal attachment [16]. Furthermore, participants were required to have no visible supra- or sub-gingival calculus and to regularly perform brushing their teeth. The study's exclusion criteria comprised individuals with periodontitis, current use of oral rinse, habitual smoking behaviour, extensive neglected dental caries, dental appliance wearers, individuals who underwent

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periodontal therapy within the past six months, those who received antibiotic therapy three months prior to the study, pregnant or breastfeeding women, and individuals with a previous record of allergic reactions to any of the items used in the investigation.

Following the process of participant selection, the objective of the inquiry was communicated to every potential participant, and written consent was procured. The decision was made to refrain from providing additional guidance on oral hygiene in order to preserve the participants' current dental hygiene routines.

Calibration

The reproducibility of the examiner's recording of clinical periodontal parameters (bleeding on probing) as determined by intra-examiner calibration. The examiner recorded periodontal parameters twice with a 2-hour interval for five participants. The acceptable limit for agreement for all clinical parameters was > 0.75. In this research, the Kappa value for parameter was 0.91.

Sample Size

To calculate sample size, IL-6 was used as a primary outcome of the study. The concentration of the biomarker in health is estimated to be equal to 6.69 ±0.89 and during gingivitis is equal to 7.85±0.82 (20). The software G*power version 3.1.9.2 was utilised to determine the appropriate sample size with a 95 % power of study, an alpha error of probability of 0.05. The results indicated that each study arm should consist of 18 participants, which includes 16 calculated individuals and an additional 2 to account for any potential dropouts. Therefore, the total sample size for the study was 54 participants.

Study Interventions

The clinical trial employed a three-arm design, utilising three different mouth rinses. The first is a positive-control rinse, consisting of 0.12% Chlorhexidine and marketed under the brand name KIN Gingival by KIN, Spain. The second rinse was a placebo, consisting of distilled water with added food colouring to ensure double-blind conditions. The third rinse, known as Perhyal, was used as the test intervention. Perhyal is manufactured by BMG Pharma in Milan, Italy and contains H_2O_2 (1.80%) and HA (0.10%) as active ingredients, with the remaining portion consisting of water (97.3%) and inert additives. The mouthwashes were stored in impermeable bottles that were uniform in appearance and were randomly labelled with letters (A, B, and C) by an individual who was not involved in the clinical trial. Therefore, maintaining volunteer and investigator blindness throughout the clinical investigation was crucial. The process of decoding was conducted subsequent to the conclusion of the inquiry. The randomization, blinding, and intervention allocation procedures were followed as reported in previous investigations [17].

Clinical Research Design

One experienced, calibrated examiner selected, enrolled, and evaluated the individuals. 54 students volunteered and agreed to the research criteria. A comprehensive intraoral examination followed. Bleeding on probing, gingival index and plaque index were assessed with sterile Williams' periodontal probes (MEDESY 546-1, Italy). Clinical examiners would clarify the study's aims and protocols if individuals met inclusion criteria. Participants who accepted the conditions and guidelines signed the informed consent form and started the investigation.

In the baseline visit at the beginning of the study period, saliva samples were taken from all 54 participants before starting with measures of the clinical parameters. The collection of unstimulated whole saliva was conducted utilizing a previously described technique [18]. The study's participants were directed to abstain from engaging in oral hygiene practices, such as brushing, flossing, and using mouth rinses, as well as consuming food, beverages, or chewing gum for a period of one hour prior to the collection of saliva samples. Subsequently, all the subjects underwent a 30-second rinse

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with 10 mL of tap water, followed by expectoration.

The participants expectorated at least 5 mL of unstimulated whole saliva in sterile containers containing lyophilized protease inhibitor solution. On the ice, saliva samples were collected. Before analysis, aliquots were prepared, and samples were frozen at -80°C .Whole saliva samples were tested for the presence of interleukin (IL)-6 and interleukin (IL)-1β by using an enzyme-linked immunoassay (ELISA) kit (USCN Business Co., Ltd., Wuhan, China), used for determining protein levels in salivary samples. The analysis was done following the manufacturer's instructions for each kit. The ELISA procedure for this study was the sandwich ELISA technique.

Then, measurement of bleeding of probing (BOP) for the six surfaces by gently introducing the periodontal probe to the level of the gingival sulcus removed coronally and waiting thirty seconds to see whether there is any bleeding (1: bleeding, 0: no bleeding) [19]. 14-days after baseline (the first visit) saliva collection and clinical periodontal parameter scoring (BOP) were repeated.

Examiners regulated mouthwash usage by giving one patient a 140mL bottle at the start of each week and asking for the returning the previous week's bottle. The mouth rinse bottles were recovered, and the remaining liquid was computed to determine usage compliance (280 ml/bottle for two weeks).

Randomization

The Simple Randomization method was used to allocate patients into three distinct groups for enrollment.

Each cohort of subjects was allocated a letter (A, B, or C) to match up with the intervention. A computer-based random number generator, implemented in Microsoft Excel 2016, was employed to generate a set of random numbers. These numbers were subsequently utilised to rearrange the order of participants (n=54) and groups, which were pre-determined. Each group was assigned an equal number of participants (n=18) in a 1:1:1 allocation. Subsequently, bottles that were appropriately labelled with the corresponding interventions were dispensed to the patients, accompanied by detailed guidelines for their administration. The participants were directed to perform a mouth rinse for a duration of one minute, using 10 mL of undiluted mouthwash, after brushing their teeth. They were also advised to refrain from consuming any food or beverage for a period of 30 minutes following the mouth rinse. In order to mitigate potential interference with the effectiveness of the interventions, participants were instructed to perform oral hygiene procedures twice a day, with a 12-hour time interval between each session. Every individual employed a toothbrush featuring filaments of a soft hardness and toothpaste from the brand COLGATE® by Colgate-Palmolive in India. The tooth-brushing method of the subjects was not allowed to be modified.

Statistical Analysis

The Statistical Package for the Social Science (SPSS) version 21, developed in Chicago, Illinois, United States of America, was utilised for the purposes of data description, analysis, and presentation. The agreement between investigators was assessed by means of Cohen's kappa coefficient. The Shapiro-Wilk test was employed to assess the normality of the distributions. To compare interventions, statistical measures such as mean and standard deviation are utilised to describe the scores of bleeding on probing %. The study utilised a parametric analysis, specifically a paired t-test, to compare the aforementioned variables within the same group.

The researchers employed a one-way analysis of variance (ANOVA) to detect potential variations among the interventions.

Subsequently, the Tukey HSD posthoc test was employed to ascertain the presence of a statistically significant distinction between every intervention pair. The Tukey HSD posthoc test was employed to determine the proportion of participants who achieved a state of good health. The effect size of the interventions in comparison to the placebo was calculated. $P < 0.05$ was

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considered to indicate statistical significance.

Results

The eligibility of 75 individuals was assessed, and 54 of them were selected following the application of inclusion and exclusion criteria. Thus, the final analysis included a total of 54 individuals. The study reports that the HA+H_2O_2 mouthwash group had a mean age of 21.22 ±0.87 years, and the Placebo group had a mean age of 21.16 ±0.92 years. The distribution of participants based on sex and treatment are shown in Table 1.

Table I: Demographic variables of the study groups.

	Study groups			
Demographic characteristics	HA/H2O2 n=18	CHX n=18	Placebo n=18	***P*-value**
Age (years)				
Range	(20-23)	(20-23)	(22-23)	0.93^{NS*}
Mean ± SD	21.11±0.83	21.2±0.87	21.16±0.92	
Sex				
Female (N)	10	10	11	0.92^{NS**}
Male (N)	8	8	6	

CHX: chlorhexidine; * one-way ANOVA test, **Chi-square test; NS: Not significant difference p > 0.05, SD: Standard deviation

Bleeding on probing

Non-significant differences (P< .05) were detected among the groups at baseline. On day 14, the mean bleeding on probing values were significantly decreased; they were 10.222 ± 0.943, 9.500 ±1.043, and 32.944 ±1.392 for HA+ H_2O_2, CHX, and Placebo, respectively, with statistically significant differences among groups. Still, the effect sizes were higher (20.478) in the HA+ H_2O_2 group than in the CHX group (15.761), with a non-significant difference between them and significant differences when each were compared to the placebo group, as shown in Table 2.

Table 2: Bleeding on probing by study groups and intervals.

	HA/ H_2O_2	0.12%CHX	Placebo	
	Mean ±SD	Mean ±SD	Mean ±SD	P value*
Baseline	34.778 ±1.309	35.000±1.188	34.556±1.149	0.553
14 days	10.222±.943	9.500 ±1.043	32.944 ±1.392	**0.000**
Paired T test	86.880	66.868	7.459	
P value**	**0.000**	**0.000**	**0.000**	
Effect size	20.478	15.761	1.758	
Multiple Comparisons				
(I) Groups	(J) Groups	Mean Difference (I-J)	P value***	
HA/H_2O_2	0.2%CHX	0.722	0.150	
	Placebo	-22.722	**0.000**	

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Interleukins -6

IL-6 levels were measured in the study groups at baseline and after 41 days, as shown in Table 3. In the HA/ H_2O_2 group, there was significant difference between mean salivary level of IL-6 before (70.956 ±3.177) pg/ML and after (29.186 ±5.716) pg/ML, with a 7.03 of effect size. In the CHX group, there was significant difference between mean salivary level of IL-6 before (71.529 ±1.485) pg/ML and after (28.118 ±2.892) pg/ML, with a 14.14 effect size. While In the placebo group, there was significant difference between mean salivary level of IL-6 before (72.09±1.29) pg/ML and after (69.50±1.05) pg/ML, with a 1.63 effect size. No significant difference between mean IL-6 changes after rinsing with HA/ H_2O_2 and CHX (P > 0.05) were seen.

Table 3: IL-6 levels by study groups and intervals.

	Groups			
	HA/ H_2O_2	**0.12%CHX**	**Placebo**	
	Mean ±SD	Mean ±SD	Mean ±SD	P value*
Baseline	70.957±3.177	71.529±1.485	72.096±1.291	0.294
14 days	29.186±5.716	28.118±2.892	69.502±1.055	**0.000**
Paired T test	29.834	59.993	6.928	
P value**	**.000**	**.000**	**.000**	
Effect size	7.032	14.140	1.633	
Multiple Comparisons				
(I) Groups	(J) Groups	Mean Difference (I-J)	P value***	
HA+ H_2O_2	0.12%CHX	1.067	0.671	
	Placebo	-40.317	**0.000**	
0.2%CHX	Placebo	-41.384	**0.000**	

*one-way ANOVA test; ** Paired T-test; *** Tukey HSD test

Interleukins -1β

IL-1β levels were measured in the study groups at baseline and after 14 days, as shown in Table 4. In the HA/ H_2O_2 group, there was significant difference between mean salivary level of IL- 1β before (181.704 ±8.018) pg/ML and after (70.013 ±1.307) pg/ML, with a 13.133 effect size. In the CHX group, there was significant difference between mean salivary level of IL-1β before (184.606 ±7.112) pg/ML and after (69.839 ±3.469) pg/ML, with a 13.549 effect size. While In the placebo group, there was significant difference between mean salivary level of IL- 1β before (182.835±5.314) pg/ML and after (171.528±5.353) pg/ML, with a 1.833 effect size. No significant differences between mean IL- 1β changes after rinsing with HA/ H_2O_2 and CHX (P > 0.05) were seen.

Table 4: IL-1β levels by study groups and intervals.

	Groups		
	HA/ H_2O_2	0.12%CHX	Placebo

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	Mean ±SD	Mean ±SD	Mean ±SD	P value*
Baseline	181.704±8.018	184.606±7.112	182.835±5.314	0.452
14 days	70.013±1.307	69.839±3.469	171.528±5.359	0.000
Paired T test	55.719	57.485	7.776	
P value**	0.000	0.000	0.000	
Effect size	13.133	13.549	1.833	

Multiple Comparisons			
(I) Groups	(J) Groups	Mean Difference (I-J)	P value***
HA/ H_2O_2	0.12%CHX	0.174	0.989
	Placebo	-101.516	0.000
0.2%CHX	Placebo	-101.689	0.000

*one-way ANOVA test; ** Paired T-test; *** Tukey HSD test

Discussion

The objective of our investigation was to assess a new composition including HA and H_2O_2 in mitigating the salivary concentrations of IL-1B and IL-6 among individuals afflicted with gingivitis caused by plaque accumulation. Both of these actives have been researched separately *in vitro* in addition to *in vivo* models, and their features are well recognized. As an instance, Rodrigues et al. [13] found that HA exhibited antibacterial properties by inhibiting the growth of two prevalent periodontopathogens, namely *Aggregatibacter actinomycetemcomitans* as well as *Prevotella intermedia*, to a comparable extent as chlorhexidine. On the other hand, a recently published randomized clinical trial conducted by Yaneva et al. [12] has provided confirmation of the debated antibacterial and anti-inflammatory effects of H_2O_2. The study's results indicated that a statistically significant decrease in salivary IL-1β and IL-6 levels in the group that used HA/ H_2O_2 mouthwash before and after use. The observed decrease in IL-1β and IL-6 concentrations may be attributed to the synergistic action of HA and H_2O_2, which are potent anti-inflammatory agents known to scavenge reactive free radicals and exhibit efficacy against both Gram-positive and Gram-negative bacterial strains. These factors are directly associated with the upregulation of cytokine levels [15,21]. As this is a new substance, being the first to investigate the effectiveness of the combination of HA and H_2O_2 on salivary cytokine levels, it is not feasible to make a direct comparison with previous studies. Nonetheless, there are further studies that have examined the impact of HA on IL-1β levels in a different kind of inflammation. Mohammad et al. [22] investigated the possibility of using HA gel application in treatment patient with periodontitis. Their results indicated that the greatest mean difference in IL-1β level was observed subsequent to the administration of hyaluronic acid gel locally in the HA group, with CHX gel application in the CHX group following closely behind. Regarding H_2O_2 is the other ingredient in the

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solution, the efficacy of H_2O_2 on cytokines in the human body has not been previously studied.
On the other hand, the present study's results indicated a statistically significant decrease in salivary IL-1β and IL-6 levels among participants who used CHX mouthwash before and after administration. This finding is consistent with prior study on the impact of CHX on gingivitis patients' IL-1β levels and IL-6 [23]. Yoshida et al. discovered that periodontal therapy, which included dental hygiene instructions, conventional mechanical treatment, and 0.12% chlorhexidine used as an adjunct for 15 days, reduced salivary IL-1B and 1l-6 in gingivitis patients [23]. IL-1β secretion requires cellular alterations or stress signals to activate inflammatory cells, which activate caspase-1 and activate IL-1β [24]. High levels of this cytokine are linked to periodontal disease's pathogenesis and progression.

Overall, IL-1β is a significant contributor to the neutrophil migration capability as it prompts the dissemination of these cells via the bloodstream. Diminishing the concentration of the aforementioned cytokine could potentially lead to a decrease in migratory requirements, as the inflammatory process would be mitigated. Through the mechanical elimination of the biofilm as a component of periodontal therapy, the difficulty was diminished, resulting in a decrease in the production of these cytokines. A recent study found the resolution of gingivitis is associated with significant reductions in both gingival inflammation and plaque levels, as well as systemic inflammatory markers [25]. In a comparison between HA/ H_2O_2 with CHX mouthwashes, the present findings revealed that CHX mouthwash had the highest effect in lowering IL-1B and IL-6 levels when compared to HA/ H_2O_2 mouthwash. Moreover, these results revealed that there was no statistically significant difference between HA/ H_2O_2 mouthwash and CHX in reducing of IL-1B levels at day 14.

The findings of our study indicated a statistically significant variance in the average bleeding on probing index percentage among all mouthwash treatments (HA/ H_2O_2, CHX, and Placebo) prior to and following a 14-day period of use. Nevertheless, the intergroup comparisons regarding bleeding on probing (BOP) during second visits did not exhibit any significant disparity between the HA/ H_2O_2 and CHX groups ($P>0.05$). The results of our study indicated a significant reduction in BOP scores when using the HA+ H_2O_2 mouthwash in comparison to both the baseline data and the negative control. This finding can be explained by anti-inflammatory properties of both constituent (HA, H_2O_2) and efficacy against both Gram-positive and Gram-negative bacterial strains of both ingredients [13,15] .The present data are compatible with Abdulkareem et al. [26], who found that a new formulation containing 0.025% HA with a 0.12% chlorhexidine mouth rinse compared to placebo resulted in a statistically significant reduction in gingival inflammation when compared to both the baseline and the placebo. Furthermore, the findings indicated that there was no significant statistical disparity between the effectiveness of HA/ H_2O_2 mouthwash and CHX in diminishing the BOP score on the 14th day.
On the other hand, the third (placebo) group in both biomarkers showed positive results. It was expected that the placebo group would also experience a decrease in gingival inflammation scores, as the patients were motivated to uphold optimal oral hygiene. The Hawthorne effect, a well-known phenomenon, could have played a role in the enhanced results observed in both cohorts. This may be attributed to an increased consciousness of oral health care [27] and the influence of the regular monitoring visits.

The trial's limitations included a short 14-day evaluation interval and the absence of instances of supra-sub calculus in the intervention group, as well as healthy controls. In addition, the trial was limited to students between the ages of 18 and 23. In addition, no effort was made to modify the subjects' diets, which

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could have had a long-term impact on the results, particularly salivary cytokines; additional research is required to determine the extent of this influence. financing.

Conclusion

Mouthwash containing HA/H_2O_2 demonstrated an anti-inflammatory effect, suggesting an immunomodulatory effect. These findings may be beneficial and encouraging for the use of HA/ H_2O_2 in the treatment of gingivitis; thus, HA/H_2O_2 rinse has the potential to serve as an appropriate replacement for chlorhexidine (CHX).

Data Availability

The data used to support the findings of this trail are available from the corresponding author upon request.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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References

1. Dental plaque–induced gingival conditions. Journal of clinical periodontology. Murakami S, Mealey BL, Mariotti A, Chapple IL. 2018 Jun;45: S17-27.
2. Evaluation of Entamoeba gingivalis and Trichomonas tenax in patients with periodontitis and gingivitis and its correlation with some risk factors. Ibrahim S, Abbas R. J Bagh Coll Dentistry. 2012;24(3):158–62.
3. Prevalence and severity of gingivitis in American adults. Li Y, Lee S, Hujoel P, Su M, Zhang W, Kim J, Zhang YP, De Vizio W. American journal of dentistry. 2010 Feb 1;23(1): 9.
4. Serum levels of interlukine-1beta and interlukine-2 in chronic periodontitis. Al-Ghurabei BH, Shaker ZF, Fadhel R, Al-Khayli NG, Mustafa LK. Al-Mustansiriya J. Sci. 2012;23(3):55-62.
5. Biologic Basis for Interleukin-l in Disease.Dinarello CA. The Journal of The American Society of Hematology. 1996. Vol. 87.
6. Interleukin-1 function and role in rheumatic disease. Schett G, Dayer JM, Manger B. Nature Reviews Rheumatology. 2016 Jan;12(1):14-24.
7. Biological effects of interleukin-6 on gingival fibroblasts: cytokine regulation in periodontitis .Naruishi K, Nagata T. Journal of cellular physiology. 2018 Sep;233(9):6393-400.
8. Effect of a chlorhexidine mouthrinse on plaque, gingival inflammation and staining in gingivitis patients: A systematic review. An Strydonck DAC, Slot DE, Van Der Velden U, Van Der Weijden F. J Clin Periodontol. 2012 Nov;39(11):1042–55.
9. Antibacterial efficiency of chlorhexidine digluconate 0.2% against oral β-hemolytic streptococci and oral Staphylococcus aureus in immunocompromisedpatients .Qanbar FH. J Bagh College Dentistry. 2012;24(2):166-9.
10. Evaluation of Salvadora persica L. and green tea anti-plaque effect: a randomized controlled crossover clinical trial. Abdulbaqi HR, Himratul-Aznita WH, Baharuddin NA. BMC complementary and alternative medicine. 2016 Dec;16(1):1-7.
11. A Systematic Review of the Effect of Oral Rinsing with H 2 O 2 on Clinical and Microbiological Parameters Related to Plaque, Gingivitis, and Microbes.Muniz FW, Cavagni J, Langa GP, Stewart B, Malheiros Z, Rösing CK. International journal of dentistry. 2020 Oct 31;2020.
12. Randomised controlled trial comparing the clinical effectiveness of mouthwashes based on essential oils, chlorhexidine, hydrogen peroxide and prebiotic in gingivitis treatment.Yaneva BK, Dermendzhieva YB, Mutafchieva MZ, Stamenov NV, Kavlakova LB, Tanev MZ, Karaslavova E, Tomov GT. Folia Medica. 2022 Aug 31;64(4):588-95.
13. Hyaluronan-containing mouthwash as an adjunctive plaque-control agent. Rodrigues SV, Acharya AB, Bhadbhade S, Thakur SL. Oral health & preventive dentistry. 2010 Jan 1;8(4):389-94.
14. Hyaluronan: its nature, distribution, functions and turnover.Fraser JR, Laurent TC, Laurent UB. Journal of internal medicine. 1997 Jul;242(1):27-33.
15. Hyaluronic acid: a boon in periodontal therapy. Dahiya P, Kamal R.North American journal of medical sciences. 2013 May;5(5):309.
16. Plaque-induced gingivitis: Case definition and diagnostic considerations. Trombelli L, Farina R, Silva CO, Tatakis DN. Journal of clinical periodontology. 2018 Jun;45:S44-67.

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Vol 11 No 1 (2023) DOI 10.5195/d3000.2023.556

17. Anti-inflammatory effects of manuka honey on salivary cytokines (clinical study). Al-Kubaisi MW, Al-Ghurabi BH, Alkubaisy W, ABDULLAH NN. Journal of Baghdad College of Dentistry. 2023 Mar 15;35(1):10-9.
18. Targeted salivary biomarkers for discrimination of periodontal health and disease (s). Ebersole JL, Nagarajan R, Akers D, Miller CS. Frontiers in cellular and infection microbiology. 2015 Aug 19;5:62.
19. Problems and proposals for recording gingivitis and plaque. Ainamo J, Bay I. International dental journal. 1975 Dec 1;25(4):229-35.
20. Changes of Four Pro-inflammatory Proteins in Whole Saliva during Experimental Gingivitis. Zhou M, Meng HX, Zhao YB, Chen ZB. Chinese Journal of Dental Research. 2012 Jan 1;15(2):121.
21. The effects of hydrogen peroxide mouthwashes on the prevention of plaque and gingival inflammation: a systematic review. Hossainian N, Slot DE, Afennich F, Van der Weijden GA. International journal of dental hygiene. 2011 Aug;9(3):171-81.
22. The Effect of Hyaluronic Acid Gel on Periodontal Parameters, Pro-Inflammatory Cytokines and Biochemical Markers in Periodontitis Patients. Mohammad CA, Mirza BA, Mahmood ZS, Zardawi FM. Gels. 2023 Apr 12;9(4):325.
23. Inflammatory markers in the saliva of cerebral palsy individuals with gingivitis after periodontal treatment. Yoshida RA, Gorjão R, Mayer MP, Corazza PF, Guare RO, Ferreira AC, Santos MTBrazilian oral research. 2019 Jul 1;33.
24. Association between interleukin-8 levels and chronic periodontal disease: A PRISMA-compliant systematic review and meta-analysis. Finoti LS, Nepomuceno R, Pigossi SC, Corbi SC, Secolin R, Scarel-Caminaga RMMedicine. 2017 Jun;96(22).
25. Treatment of gingivitis is associated with reduction of systemic inflammation and improvement of oral health-related quality of life: A randomized clinical trial. Perić M, Marhl U, Gennai S, Marruganti C, Graziani F. Journal of Clinical Periodontology. 2022 Sep;49(9):899-910.
26. A randomized double-blind clinical trial to evaluate the efficacy of chlorhexidine, antioxidant, and hyaluronic acid mouthwashes in the management of biofilm-induced gingivitis. Abdulkareem AA, Al Marah ZA, Abdulbaqi HR, Alshaeli AJ, Milward MR International journal of dental hygiene. 2020 Aug;18(3):268-77.
27. The" Hawthorne effect"—what did the original Hawthorne studies actually show? Wickström G, Bendix T. Scandinavian journal of work, environment & health. 2000 Aug 1:363-7.