

# Comparative Study Of Clinical Efficacy Of Chlorhexidine And Chlorhexidine Plus Hyaluronic Acid Mouthwashes In Patients Of Chronic Periodontitis

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## Abstract

**Background:** The use of mouthwashes is an irreplaceable aspect of chemical plaque control due to its efficacy, ease of use and patient acceptance. Although chlorhexidine has been considered the gold standard over the years, the efficacy of hyaluronic acid is yet to be substantiated.

**Aim:** The aim of this study is to evaluate and compare the clinical efficacy of chlorhexidine versus chlorhexidine plus hyaluronic acid mouthwashes in patients of chronic periodontitis.

**Method:** 68 patients, diagnosed with generalized chronic periodontitis, enrolled in study after fulfilling the inclusion criteria, underwent scaling and root planing (SRP). After which, patients in the control group (n=34) were prescribed 0.2% chlorhexidine mouthwash while patients in the test group (n=34) were prescribed 0.2% chlorhexidine plus 0.1% hyaluronic acid mouthwash after SRP. All clinical parameters were assessed at baseline (T0) prior to SRP and then at 14 days (T1), except stain index which was again evaluated at 28 days (T2).

**Result:** After the comparative evaluation of the clinical parameters between the two groups at baseline and at 14 days, the results substantiated that the patients using chlorhexidine plus hyaluronic acid mouthwashes demonstrated significant improvement in terms of reduction in pocket probing depth, plaque, gingival and bleeding index while no significant intergroup differences could be appreciated in terms of the stain index at 28 days.

**Conclusion:** It was concluded that the heightened efficacy of hyaluronic acid is due to its anti-inflammatory, antioxidative, bacteriostatic properties as well as its hygroscopic and biocompatible nature.

**Keywords:** Chemical plaque control, Chlorhexidine, Hyaluronic acid

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## I. Introduction

Scaling and root surface debridement comprises of the thorough cleaning of the tooth and root surfaces to remove dental plaque and calculus from periodontal pockets as well as to smoothen the root to remove bacterial toxins. However, conventional debridement procedures do not remove all periodontopathic bacteria from the

subgingival environment, especially those in inaccessible areas such as furcations, grooves, concavities, and deep pockets. [1] Therefore, chemotherapeutic agents are imperative as an adjunct to control the progression of gingival diseases as bacteria present in the soft tissues can re-colonize on tooth surfaces even after mechanical plaque control [2].

Chlorhexidine digluconate was discovered by The Imperial Chemical Industries, Ltd, Manchester, United Kingdom in the 1950s. It is a potent broad-spectrum antiseptic agent, [3] and has been considered the gold standard amongst antiplaque agents due to its heightened substantivity in the oral cavity, to both hard and soft tissues, lasting for about twelve hours after its use. [4] Chlorhexidine chemically is a bisbiguanide and di-cation that raises the salivary pH, exerts a buffering action and increases the oxygen saturation, thereby reducing the proportion of pathogenic bacteria by altering the ecosystem of periodontal pockets. When used topically as a mouth rinse, the N-chlorinated derivative of chlorhexidine binds covalently to the proteins present in the oral mucosa resulting in a persistent antimicrobial effect with limited systemic absorption [5].

Hyaluronic acid (HA) is a naturally occurring linear polysaccharide of the extracellular matrix of connective tissue, synovial fluid, and other tissues made up of repeating units d-glucuronic acid and N-acetyl-d-glucosamine. [6] According to a study by Rodrigues. al, the use 0.025% hyaluronan mouthwash distinctively suppressed the growth of *Aggregatibacter actinomycetemcomitans* (Aa) and *Prevotella intermedia*, and showed beneficial effects in accelerating healing and reducing pocket depth as a result of its physicochemical anti-inflammatory, anti-edematous, and anti-oxidant properties along with its non-antigenic and biocompatible nature. [7]

Although chlorhexidine has been considered as the benchmark amongst the antiplaque agents, due to the numerous anti-inflammatory properties of hyaluronic acid, various studies are being conducted to substantiate the synergistic effect of both.

## **II. Materials And Method**

Sample size was based on the power of test which is 80% with the level of significance being 5%. 68 patients, willing to participate in the study and appear for follow up appointments were enrolled in the study, after fulfillment of the inclusion and exclusion criteria. The study was conducted after attaining approval from the Institutional Review Board and the Ethical Committee in accordance to the Helsinki Declaration of 1975, as revised in 2000. The inclusion criteria comprised of patients aged between 18 to 55 years, and diagnosed with Stage II and Stage III dental biofilm induced generalized chronic periodontitis (World Workshop 2017) i.e., pocket probing depth more than or equal to five mm and interdental clinical attachment loss greater than or equal to five mm. [8]

The exclusion criteria for the study comprised of patients having intrinsic stains, known systemic disease or on any medication known to interfere with outcomes of periodontal therapy, pregnant or lactating women, individuals with any habit of smoking or chewing of tobacco, betel nut or pan masala, and those who have undergone scaling and root planing or any form of periodontal therapy within the last six months.

### **Randomization and allocation**

Participants were randomly allocated into two groups: test and control using the coin toss method. Blinding of both the operator and the participant was performed. All the scaling and root planing procedures were carried out by a single operator while the assessment of the clinical parameters was performed by another examiner. The mouthwashes were removed from their packaging, and wrapped in different colored paper to ensure blinding of the participant. As a part of the blinding protocol, the patients were given the designated mouthwash by a separate investigator.

### **Clinical procedure**

The clinical parameters were assessed at baseline before scaling and root planing and then at 14 days, except for stain index which was evaluated again at 28 days. The patients underwent scaling and root planing, after which they were given oral hygiene instructions. Patients were advised to brush their teeth using a toothbrush and were demonstrated the appropriate brushing technique by the operator. Patients in both the groups were instructed to use the allotted mouthwash ten ml twice daily for 28 days.

### **Patient assessment**

The patients were assessed for the following clinical parameters at baseline and at 14 days, except the Stain Index which was evaluated at 28 days follow up also.

1. Plaque Index (Silness and Loe 1964) [9]
2. Gingival Index (Loe and Silness 1963) [9]
3. Modified Sulcular Bleeding Index (Mombelli et al.1987) [10]
4. Periodontal pocket probing depth

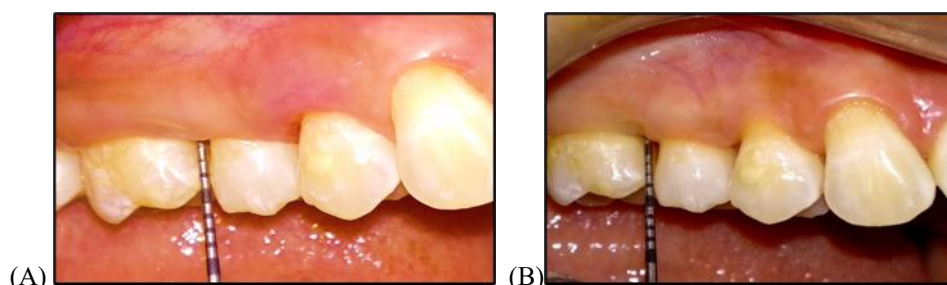
5. Macpherson Modification of Lobene’s Stain Index (2000) [11]

**Statistical Analysis**

The collected data was entered in Microsoft excel spreadsheet. The entire data is statistically analyzed using Statistical Package for Social Sciences (SPSS version 26.0, IBM Corporation, USA) for MS Windows. The Shapiro-Wilk test was used to check the normality of the continuous variable.

- Unpaired t-test/Mann-Whitney U test was used to check significant difference between two independent sample.
- Paired t-test/Wilcoxon Sign Rank test was used to check significant difference on dependent sample.
- Three repeated groups were compared by Friedman test.

Probabilities of greater than 0.05 considered as statistically not significant whereas probabilities less than 0.05 and 0.01 were accepted as statistically significant and highly significant respectively.



**FIG.1 (A)** Baseline probing depth 6 mm **(B)** Probing depth 4 mm after 14 days in patient using chlorhexidine plus hyaluronic acid mouthwash

**III. Results**

68 patients participated in the study with 34 patients each in both the control and test group. The sample comprised of systemically healthy patients, with no habit of tobacco chewing or smoking, and who had not undergone any form of periodontal therapy in the last six months. Group A comprised of patients in the control group who were prescribed only chlorhexidine mouthwash post oral prophylaxis, while Group B comprised of patients in the test group who were prescribed chlorhexidine plus hyaluronic acid mouthwash.

Although statistically significant changes were observed in terms of reduction in periodontal pocket depth in both the groups (Fig.1), no noteworthy intergroup difference was seen in both the groups (Graph 1). While notable reduction in the plaque (Table 1) and gingival scores (Table 2) can be appreciated in both the groups from baseline to 14 days, the mean differences in their scores are 0.26 and 0.30 respectively, which is clinically insignificant. The P-value in the bleeding index scores for both the groups are <0.001 which is clinically significant. Moreover, remarkable inter group difference is substantiated with a mean difference of 5.77 in the bleeding scores at the 14<sup>th</sup> day evaluation (Graph 2). With respect to the staining index, while staining scores increased slightly from 14<sup>th</sup> day to 28<sup>th</sup> day in both the groups, notable reduction in the stain scores were seen in the test group patients with a mean difference of 3.23 on the 28<sup>th</sup> day (Graph 3).

**TABLE 1:** Comparison of Group A and Group B with respect to Plaque Index at Baseline and Day 14

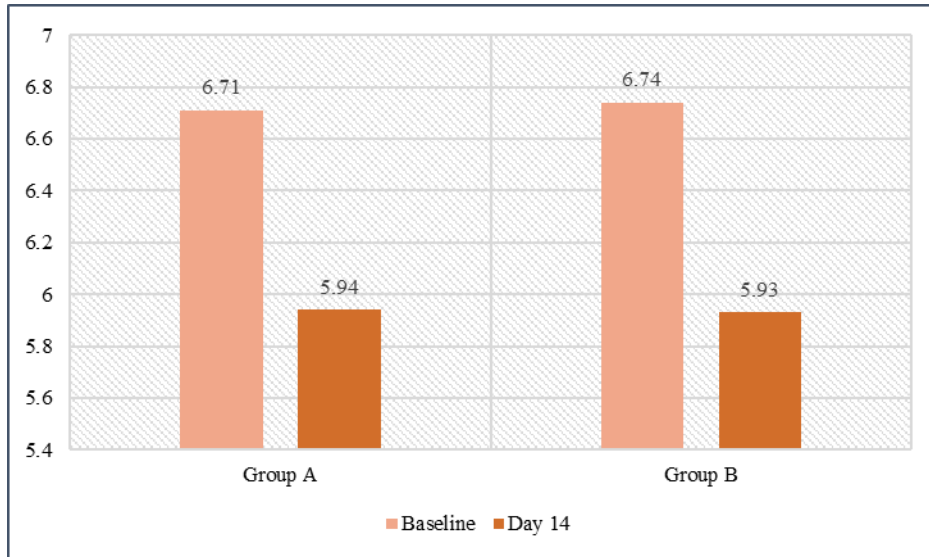
Evaluation	Group A(n=34)		Group B(n=34)		Mean Difference	P-value*
	Mean	SD	Mean	SD		
Baseline (T0)	2.61	0.36	2.49	0.44	0.12	0.254
Day 14 (T1)	0.69	0.46	0.43	0.31	0.26	0.014
Pair Difference	1.92		2.06			
P-value †	<0.001		<0.001			

\*: Mann-Whitney U test, †: Wilcoxon Sign Rank test

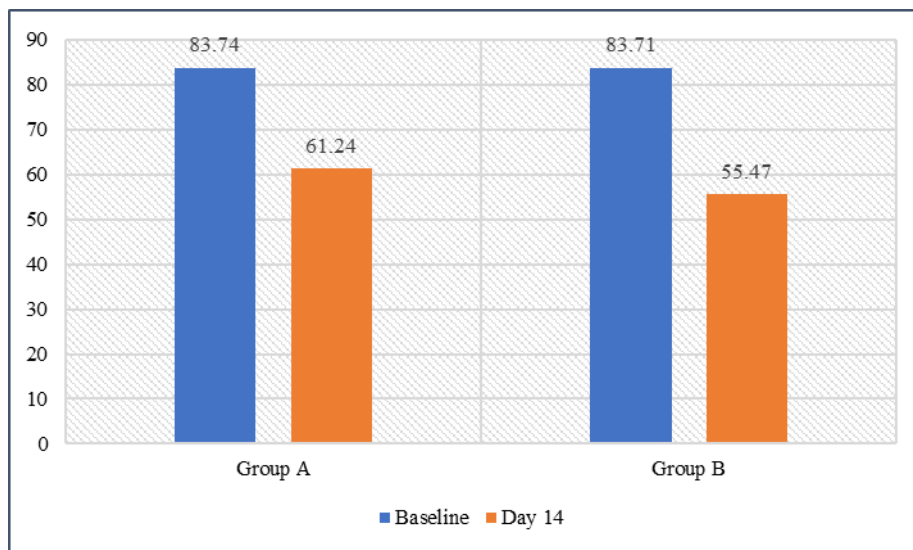
**TABLE 2:** Comparison of Group A and Group B with respect to Gingival Index at Baseline and Day 14

Evaluation	Group A(n=34)		Group B(n=34)		Mean Difference	P-value*
	Mean	SD	Mean	SD		
Baseline (T0)	2.39	0.47	2.26	0.59	0.13	0.506
Day 14 (T1)	1.02	0.45	0.72	0.46	0.30	0.006
Pair Difference	1.37		1.54			
P-value †	<0.001		<0.001			

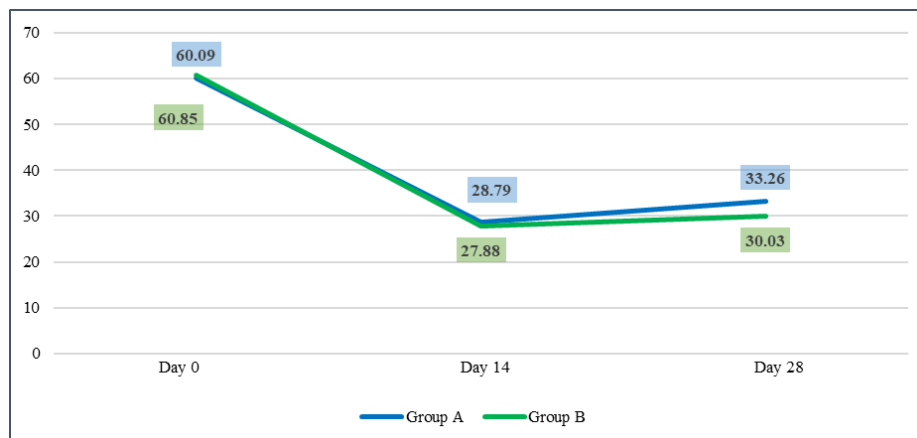
\*: Mann-Whitney U test, †: Wilcoxon Sign Rank test



**GRAPH 1:** Mean Pocket Probing Depth at T0 and T1 of Group A & Group B.



**GRAPH 2:** Mean Bleeding Index at T0 and T1 of Group A & Group B



**GRAPH 3:** Mean Staining Index at T0, T1 and T2 of Group A & Group B

#### **IV. Discussion**

The current study was designed to evaluate the synergistic effect of hyaluronic acid in a mouthwash on the periodontal status of patients with chronic periodontitis and to compare its efficacy with the standard dosage

of chlorhexidine (0.2%) through the evaluation of various periodontal parameters like plaque index (PI), gingival index (GI), bleeding index, stain index, and periodontal probing depth after 14 days of scaling and root planing (SRP).

Periodontitis is a complex multifactorial inflammatory pathology that has been established as the sixth most globally prevalent non-communicable disease, estimated to affect 11.2% of the global adult population, hence representing a significant healthcare, social, and economic burden [12]. The oral biofilm is the primary etiologic factor implicated in the initiation and progression of the disease, if left untreated. Therefore, the elimination of the biofilm that harbors innumerable periodontopathic bacteria, is considered as the prime requisite in the resolution of periodontitis.

In the existing study, we have focused on the ability of hyaluronic acid to restore the health of gingiva through its anti-inflammatory and bacteriostatic properties in adjunct to chlorhexidine and mechanical debridement. This was in compliance with data obtained from a recent study conducted by Aryan A. Sabri et al., elicits similar results wherein 0.025% hyaluronic acid mouthwash usage demonstrated significant reductions in bleeding on probing, plaque and gingival index, and probing depth. [13]

According to Dahiya et al., hyaluronic acid can be considered as a boon in periodontal therapy due to its contribution in tissue healing by preventing periodontal pathogen colonization and degradation of the extracellular matrix proteins by serine proteases whilst promoting angiogenesis and cell proliferation. [3] Hakansson et al. suggested the role of hyaluronan in chemotactic migration and adherence of polymorphonuclear leukocytes and macrophages at the inflamed site and the phagocytosis and killing of invading microbes. Such events allow the counteraction of colonization and proliferation of anaerobic pathogenic bacteria in the gingival crevice and adjacent periodontal tissues, facilitating in the healing process. [14]

A study conducted by Genovesi et al., aimed to evaluate similar periodontal parameters as taken in the current study except in patients after implant placement. The study substantiated that the incidence of edema was significantly lower two days post-surgery in patients who were prescribed with chlorhexidine plus hyaluronic acid compared to chlorhexidine rinse. [15]

From an aesthetic perspective, one of the most unsolicited outcomes of prolonged chlorhexidine usage is the pigmentation of teeth, attributed to two physiologic processes: protein denaturation leading to metal sulphides formation; and the Maillard reaction, which develops brown staining substances, known as melanoidins. [16,17] Various anti-discoloration systems (ADS) have been developed to reduce chlorhexidine pigmentation such as sodium-metabisulphite, ascorbic acid, and cetylpyridinium chloride (CPC) [18]. However, as substantiated in a study by Guerra, addition of ADS decreases chlorhexidine's efficacy in reducing plaque and bleeding scores, but results in greater patient acceptance. [19] In the current study, although no substantial difference was noted in the staining effect with the addition of hyaluronic acid, there were no antagonistic effects on other clinical outcomes like probing depth and bleeding due to the presence of the same.

However, in contrast to our study, Saleh et al. demonstrated no remarkable difference between hyaluronic acid and chlorhexidine mouthwashes when prescribed to patients in the treatment of orthodontically induced gingival enlargement. [20] Yet, the use of hyaluronic acid as a mouthwash can prove to be beneficial as it overcomes the adverse effects of alteration of taste, burning sensation, mucosal irritation and primarily discoloration of teeth as seen with usage of chlorhexidine mouthwash.

## **V. Limitations And Future Scope Of Study**

The stain index is a highly variable parameter that is influenced by numerous factors like the patient's diet, age etc. and can influence the outcomes of the study, and deliberation of these confounding factors should be taken priorly. Moreover, use of spectrophotometric evaluation can reduce the subjective perception of staining on the tooth surfaces. [18] The comparison of chlorhexidine and hyaluronic acid mouthwashes can be extended to patients after undergoing periodontal surgery or implant placement to assess their effect on healing. Parameters such as patient's perception in terms of dryness of mouth, burning sensation, alteration in taste, which are frequent complaints in the usage of chlorhexidine can also be taken into consideration in forthcoming studies to enhance the outcomes of the study.

## **VI. Conclusion**

The role of SRP in the eradication of the biofilm is imperative. However, the adjunctive use of chemical means in plaque control offers a harmonious reduction of bacterial load and the elimination of periodontopathic bacteria in the biofilm. Both chlorhexidine and chlorhexidine plus hyaluronic acid mouthwashes led to a decline in plaque, gingival, bleeding scores and pocket depth. However, patients using the chlorhexidine plus hyaluronic acid mouth rinse showed more significant reduction in all the parameters compared to chlorhexidine alone. Moreover, the addition of hyaluronic acid reduced the adverse discoloring effect of chlorhexidine mouth rinses, although this was appreciated minimally. Thus, through the current study, it can be implicated that the use of hyaluronic acid in mouthwashes is beneficial for the healing of periodontal tissues after SRP and that it can be

routinely prescribed in clinical practice. With this, the study also aims to emphasize on the importance of simultaneous use of chemical plaque control in adjunction to mechanical plaque control in the treatment of periodontal diseases.

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