Section : Research Paper
ISSN 2063-5346

EFFICACY OF HYALURONIC ACID AND CHLORHEXIDINE MOUTHWASH IN PERIODONTITIS PATIENTS: A CLINICO-MICROBIOLOGICAL STUDY

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ABSTRACT:

Background: Periodontitis is an infectious condition that causes inflammation of the tissues surrounding and protecting the teeth. It leads to the gradual breakdown of connective tissue, loss of alveolar bone, and in severe cases, tooth loss. Nonsurgical mechanical therapy serves as the cornerstone of periodontal treatment, playing a fundamental role in managing the disease and its progression.

Aim: To evaluate the efficacy of Hyaluronic acid in combination with chlorhexidine and chlorhexidine mouthwash as an adjunct to Phase-I therapy in periodontitis patients.

Methodology: A total of 10 periodontitis patients were enrolled and randomly divided into 2 groups of 5 each by coin toss method. Group A and group B were treated with 0.025% hyaluronic acid+Chlorhexidine gluconate 0.2% and chlorhexidine gluconate 0.2%, respectively, along with phase I therapy. Patients were directed to use 10ml of the respective mouthwash, twice daily for 21 days. A pre-oral and post-oral saline rinse of 5ml each was collected at the baseline and on the 21st day and sent immediately to the microbiological laboratory for semi-quantitative analysis. Clinical parameters such as Gingival Index (GI), Plaque Index (PI), Papillary Bleeding Index (PBI), Periodontal Pocket depth (PPD), and Clinical attachment loss (CAL) were recorded at baseline, on the 7th day, and on the 21st day after phase – I therapy.

Result: In both the groups a significant reduction in PI, GI, BI and CFU were observed between baseline and after 21 days of phase- I therapy ($p<0.05$)

Conclusion: Using hyaluronic acid (HA) and chlorhexidine (CHX) mouthwashes alongside SRP improves periodontal health in patients with periodontitis, with HA having a stronger effect.

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Keywords: Periodontitis, hyaluronic acid, chlorhexidine, mouthwash, Phase-I therapy, adjunctive treatment, clinical parameters, microbiological analysis.

INTRODUCTION:

According to Shaddox and Walker (2010)[1], periodontitis is a common oral disease characterized by chronic inflammation of the periodontal tissues, which is caused by the accumulation of excessive amounts of dental plaque.

In advanced cases, periodontitis can lead to tooth mobility, occasional pain, discomfort, compromised ability to chew food, and eventual tooth loss (Charles, 2008)[2]. The main objectives of periodontal therapy, as stated by Plessas (2014)[3], are to eliminate inflammation, halt the progression of periodontal disease, improve esthetics, and establish an environment conducive to the maintenance of periodontal health. Drisko (2001)[4] stated that, scaling and root surface debridement, which involves thorough cleaning of the root surfaces, is an effective method for treating and controlling periodontal disease. It aims to remove dental plaque and calculus from periodontal pockets while also smoothening the tooth root to eliminate bacterial toxins. However, Tanwar et al. (2016)[5] pointed out that conventional mechanical debridement procedures may not completely eliminate all periodontopathic bacteria from the subgingival environment, particularly in inaccessible areas such as furcation, grooves, concavities, and deep pockets.

According to Aghili et al. (2015)[6], chlorhexidine (CHX) digluconate is widely recognized as one of the most commonly used compounds. Since the 1950s, it has served as a potent broad-spectrum antiseptic agent in medicine, exhibiting a significant antimicrobial effect against both Gram-negative and Gram-positive bacteria, as well as certain fungi and viruses. Additionally, CHX has been shown to have the ability to inhibit the formation and development of bacterial plaque for several hours, which was demonstrated in the 1970s due to its strong affinity for oral surfaces.

Hyaluronic acid (HA) has garnered increasing attention in recent years for its potential in treating inflammatory conditions across various medical fields, including dentistry, dermatology, ophthalmology, and orthopedics (Bansal et al., 2010)[7]. A novel product called hyaluronan (HA) mouthwash has been developed, containing a high molecular weight fraction of HA produced through a biotechnological process that does not involve animals. HA, derived from two repeating disaccharide subunits, is a natural component of the body's glycosaminoglycan (GAG) population. Its non-toxicity, biocompatibility, and

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diverse biochemical and physiochemical properties make it suitable for topical application in periodontal pockets, potentially promoting accelerated healing and reduced pocket depth (Moseley et al., 2002)[8].

While numerous studies have focused on the effects of non-surgical periodontal therapy, such as scaling and root surface debridement, on serum inflammatory parameters in periodontitis, there is a noticeable gap in the literature regarding the healing effects of hyaluronic acid (0.025%) mouthwash compared to chlorhexidine (0.12%) in treating periodontitis patients. This study aims to address this gap by examining the clinical and microbiological parameters to determine the efficacy of hyaluronic acid mouthwash as a potential therapeutic intervention.

AIM OF THE STUDY:

To assess the clinical and microbiological effects of mouthwashes containing hyaluronic acid (0.025%) in combination with chlorhexidine and chlorhexidine (0.12%), used in combination with scaling and root planing, in patients with periodontitis.

MATERIALS AND METHODS:

Setting of the study: The study was conducted at the Department of Periodontics at Anil Neerukonda Institute of Dental Sciences. A single trained dentist performed the study.

Inclusion Criteria:

1. Age: Participants aged between 25 and 55 years.

2. Periodontal Condition: Patients diagnosed with mild to severe periodontitis.

3. Minimum Tooth Presence: Participants with a minimum of 8 teeth.

4. Probing Depths: Probing depths (PDs) equal to or greater than 4mm.

5. Clinical Attachment Loss: Clinical attachment loss equal to or greater than 2mm.

Exclusion Criteria:

1. Smoking: Patients who are smokers.

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2. Systemic Compromises: Individuals with systemic conditions that may compromise periodontal health.

3. Medications: Patients currently taking medications, such as corticosteroids or calcium channel blockers, known to interfere with periodontal wound healing.

4. Pregnancy and Lactation: Pregnant or lactating women.

5. Recent Periodontal Therapy: Patients who have undergone periodontal therapy within the past 6 months.

These exclusion criteria are important to ensure that the study focuses on a specific population and minimizes confounding factors that could affect the outcomes of the evaluation.

Methodology: A total of 10 patients with periodontitis were included in the study. They were randomly divided into two groups, Group A and Group B, consisting of 5 patients each. Group A received treatment with a 0.025% hyaluronic acid mouthwash, while Group B received a 0.2% chlorhexidine mouthwash. Both groups underwent phase I therapy as part of their treatment regimen.

At the baseline and on the 21st day, before and after rinsing with saline, 5ml of oral rinse was collected from each patient. These samples were immediately sent to the microbiological laboratory for semi-quantitative analysis.

Various clinical parameters, including Gingival Index (GI), Plaque Index (PI), Papillary Bleeding Index (PBI), Periodontal Pocket depth (PPD), and Clinical Attachment Loss (CAL), were recorded at baseline, on the 7th day, and on the 21st day after completion of phase I therapy. These parameters were used to evaluate the effectiveness of the combined therapy with hyaluronic acid and chlorhexidine mouthwashes in improving periodontal health in patients with periodontitis.

During the study, the participants were instructed to use 10 ml of undiluted mouthwash twice daily. They were advised to rinse their mouth with the mouthwash for a duration of 1 minute. The mouthwash was to be used 30 minutes after tooth brushing, and participants were instructed to avoid eating or brushing their teeth for 30 minutes after rinsing.

In addition to the mouthwash regimen, the participants were also taught and instructed to follow the Modified Bass tooth brushing technique at the baseline.

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Statistical analysis: A comparison of clinical parameters was conducted at three time points: baseline, 7th day, and 21st day. The clinical parameters evaluated included Gingival Index (GI), Plaque Index (PI), Papillary Bleeding Index (PBI), Periodontal Pocket depth (PPD), and Clinical Attachment Loss (CAL). Paired t-tests were used to analyze the data and determine any significant changes in these clinical parameters over time.

Additionally, microbiological analysis was performed at baseline and on the 21st day. A comparison between the two groups, HA (Hyaluronic Acid) and CHX (Chlorhexidine), was conducted using an independent t-test. The purpose of this analysis was to determine if there were any significant differences in the outcomes between the two groups.

RESULTS:

Comparison of clinical and Microbiological parameters in HA and CHX

The clinical and microbiological parameters were compared between the HA (hydroxyapatite) and CHX (chlorhexidine) groups. In the GI (gingival index) parameter, the baseline values were 1.57 ± 0.58 in the HA group and 1.96 ± 0.38 in the CHX group. After 7 days, the values decreased to 0.75 ± 0.46 in the HA group and 1.59 ± 0.45 in the CHX group. At 21 days, the values further decreased to 0.42 ± 0.16 in the HA group and 1.49 ± 0.46 in the CHX group. The P value for this parameter was found to be statistically significant at 0.001, indicating a significant difference between the two groups.

For the PI (plaque index) parameter, the HA group had baseline values of 2.04 ± 0.63, which reduced to 1.12 ± 0.46 after 7 days and further to 0.50 ± 0.26 after 21 days. Similarly, the CHX group had baseline values of 2.02 ± 1.37, which decreased to 1.62 ± 0.31 after 7 days and 1.44 ± 0.27 after 21 days. The P value for the PI parameter was 0.04, indicating a statistically significant difference between the groups.

In the PBI (plaque bleeding index) parameter, the HA group had baseline values of 3.20 ± 0.44, which reduced to 2.0 ± 0.7 after 7 days and further to 1.20 ± 0.44 after 21 days. The CHX group had baseline values of 2.40 ± 1.14, which decreased to 2.21 ± 1.14 after 7 days and 1.80 ± 0.44 after 21 days. The P value for the PBI parameter was 0.04, indicating a statistically significant difference between the groups.

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Regarding the periodontal parameters, the PPD (probing pocket depth) measured in millimeters showed values of 4.40 ± 0.54 in the HA group and 2.80 ± 0.37 in the CHX group at baseline. After 7 days, the values were 3.0 ± 0.7 in the HA group and 1.80 ± 0.37 in the CHX group. At 21 days, the values were 2.60 ± 1.51 in the HA group and 2.40 ± 0.89 in the CHX group. The P value for PPD was 0.136, indicating no statistically significant difference between the groups.

The CAL (clinical attachment level) parameter, also measured in millimeters, showed consistent values throughout the study. The HA group had baseline values of 2.20 ± 0.83, which remained unchanged at 2.20 ± 1.30 after 7 days and 2.20 ± 0.83 after 21 days. Similarly, the CHX group had baseline values of 2.20 ± 1.30, which remained stable at 2.0 ± 1.0 after 7 days and 1.60 ± 0.54 after 21 days. The P value for CAL was 0.334, indicating no statistically significant difference between the groups.

Lastly, the microbiological parameter, measured as MC AGAR MEDIUM (CFU / ML), showed a significant difference between the HA and CHX groups. The HA group had baseline values of 2.80 ± 0.54, which decreased to 1.40 ± 1.09 after the study period. On the other hand, the CHX group had baseline values of 2.40 ± 0.54, which remained relatively stable. The P value for the microbiological parameter was 0.001, indicating a statistically significant difference between the groups.

In summary, the clinical and microbiological parameters demonstrated varying responses between the HA and CHX groups, with significant differences observed in the GI, PI, PBI, and microbiological parameters. However, no significant differences were observed in the PPD and CAL parameters between the groups.

Variable	**Group**	**Baseline**	**7 days**	**21 days**	**P value**
GI	HA	1.57 ± 0.58	0.75 ± 0.46	0.42 ± 0.16	**0.001 ***
	CHX	1.96 ± 0.38	1.59 ± 0.45	1.49 ± 0.46	
PI	HA	2.04 ± 0.63	1.12 ± 0.46	0.50 ± 0.26	**0.04 ***
	CHX	2.02 ± 1.37	1.62 ± 0.31	1.44 ± 0.27	
PBI	HA	3.20 ± 0.44	2.0 ± 0.7	1.20 ± 0.44	**0.04***
	CHX	2.40 ± 1.14	2.21 ± 1.14	1.80 ± 0.44	

PPD (mm)	HA CHX	4.40 ± 0.54 2.80 ± 0.37	3.0 ± 0.7 1.80 ± 0.37	2.60 ± 1.51 2.40 ± 0.89	0.136
CAL (mm)	HA CHX	2.20 ± 0.83 2.20 ± 1.30	2.20 ± 0.83 2.0 ± 1.0	1.60 ± 0.54 2.20 ± 1.30	0.334
MC AGAR MEDIUM (CFU / ML)	HA CHX	2.80 ± 0.54 2.40 ± 0.54		1.40 ± 1.09 2.20 ± 0.83	**0.001 ***

INTER GROUP COMPARISON OF CLINICAL AND MICROBIOLOGICAL PARAMETERS

The inter-group comparison of clinical and microbiological parameters between the HA group and the CHX group revealed interesting findings.

Firstly, in terms of the Gingival Index (GI), the HA group had a higher mean value of 1.68 ± 0.45, while the CHX group exhibited a lower mean value of 0.91 ± 0.64. This difference was statistically significant (p = 0.001 *), indicating that the HA group had better gingival health compared to the CHX group.

Moving on to the Plaque Index (PI), the HA group had a mean value of 1.69 ± 0.34, slightly higher than the CHX group's mean value of 1.21 ± 0.78. However, this difference was still significant (p = 0.04 *), suggesting that the HA group had a higher level of plaque accumulation compared to the CHX group.

The Bleeding Index (BI) also demonstrated significant differences between the two groups. The HA group had a mean value of 2.67 ± 0.90, while the CHX group had a slightly lower mean value of 2.13 ± 0.99. This difference was statistically significant (p = 0.04 *), indicating that the HA group experienced less bleeding during the examination compared to the CHX group.

Regarding the Probing Pocket Depth (PPD), no significant difference was observed between the HA and CHX groups. The HA group had a mean value of 2.33 ± 0.90, and the CHX group had a mean value of 2.17 ± 1.23 (p = 0.136), suggesting that both groups had similar pocket depths.

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Similarly, there was no significant difference in Clinical Attachment Loss (CAL) between the HA and CHX groups. The mean values for the HA and CHX groups were 2.13 ± 1.12 and 1.80 ± 0.67, respectively (p = 0.334), indicating that both groups experienced similar levels of attachment loss.

Lastly, the Microbiological analysis using MC Conkey Agar Medium (CFU/ML) revealed a significant difference between the HA and CHX groups. The HA group had a higher mean value of 2.60 ± 0.84, while the CHX group had a lower mean value of 1.40 ± 0.156 (p = 0.001 *), indicating that the HA group had a higher bacterial load compared to the CHX group.

S.NO	VARIABLE	GROUP HA	GROUP CHX	P VALUE
1	GINGIVAL INDEX	1.68 ± 0.45	0.91 ± 0.64	**0.001 ***
2	PLAQUE INDEX	1.69 ± 0.34	1.21 ± 0.78	**0.04 ***
3	BLEEDING INDEX	2.67 ± 0.90	2.13 ± 0.99	**0.04 ***
4	PROBING POCKET DEPTH	2.33 ± 0.90	2.17 ± 1.23	0.136
5	CLINICAL ATTACHMENT LOSS	2.13 ± 1.12	1.80 ± 0.67	0.334
6	MC CONKEY AGAR MEDIUM (CFU/ML)	2.60 ± 0.84	1.40 ± 0.156	**0.001 ***

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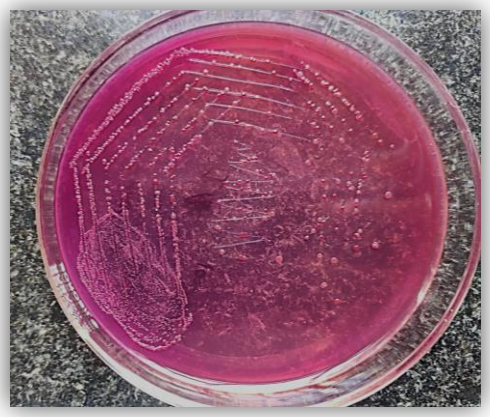
GROUP HA



BASELINE **21ST DAY**

GROUP CHX

BASELINE **21ST DAY**

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DISCUSSION:

The beneficial effects of Hyaluronic Acid (HA) in the field of periodontology have been extensively studied. HA exhibits anti-inflammatory and anti-edematous properties, which contribute to tissue healing, as demonstrated by Jentsch et al. in 1964[9]. This suggests that HA can aid in the recovery and regeneration of damaged tissues.

In addition to its anti-inflammatory effects, HA has been found to possess antioxidative properties. Dahiya et al. (2013)[10] reported that HA scavenges reactive oxygen species, thereby stabilizing the granulation tissue matrix. This antioxidant activity further supports the role of HA in promoting tissue healing.

The physical properties of HA make it an effective barrier against bacteria and their products in the extracellular matrix, as observed by Cortivo et al. in 1986. This highlights the potential of HA as a protective agent against bacterial invasion and subsequent tissue damage.

Another significant finding is the chymotrypsin-induced binding of HA to Treponema denticola, which prevents the destructive action on the periodontium, as described by Haapasalo et al. in 1996[11]. This interaction between HA and specific pathogens suggests a potential therapeutic role for HA in inhibiting the progression of periodontal diseases.

Furthermore, Pomowski et al. (2003)[12] demonstrated that HA enhances the formation of an extracellular connective tissue matrix, leading to non-inflamed and controlled bleeding. This supports the notion that HA can contribute to the restoration of healthy periodontal tissues.

It is worth noting that HA is considered biocompatible and safe for use, with no evidence of cytotoxicity reported by Campoccia et al. in 1998[13]. This reinforces the potential of HA as a clinically viable treatment option in periodontal therapy.

In terms of clinical application, the adjunctive use of HA in conjunction with scaling and root surface debridement (S&RSD) has shown promising results. Jentsch et al. (Journal of periodontology) observed a significant improvement in gingival and periodontal health following the application of HA as an adjunct to S&RSD in the treatment of gingivitis. Similarly, Johannsen et al. (2009)[14] reported that the local application of hyaluronan gel in combination with

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S&RSD may have a beneficial effect on periodontal health in patients with chronic periodontitis.

Contrasting findings were reported by Xu et al. in 2004[15], where no significant clinical or microbiological improvement was observed with the adjunctive use of HA gel compared to S&RSD alone. This discrepancy may suggest variations in treatment response among individuals or the presence of specific influencing factors.

A study conducted by Aryan A. Sabri and Saeed A. Mohammed (Journal of University of Duhok)[16] compared the use of hyaluronic acid and chlorhexidine mouthwash as adjuncts to scaling and root surface debridement in chronic periodontitis patients. Their findings revealed a significant beneficial effect of hyaluronic acid on periodontal health compared to chlorhexidine, further supporting the potential of HA in managing chronic periodontitis.

In conclusion, the data discussed above collectively highlight the diverse therapeutic properties of HA in periodontal health. While the anti-inflammatory, antioxidative, and physical barrier effects of HA demonstrate its potential in promoting tissue healing and preventing tissue damage, the clinical application of HA as an adjunct to S&RSD shows promise in improving gingival and periodontal health. However, the varying outcomes reported in different studies emphasize the need for further research to optimize treatment protocols, identify patient-specific factors, and enhance our understanding of the mechanisms underlying the effects of HA in periodontal therapy.

CONCLUSION: Effective treatment of periodontal disorders requires a drug with combined antibacterial and healing properties. It is anticipated that hyaluronic acid (HA) will emerge as the leading contender in this competition in the foreseeable future.

LIMITATIONS:

The present study acknowledges several limitations that may impact the reliability and generalizability of its findings. Firstly, the sample size employed in this research is small, which may limit the ability to draw definitive conclusions and make broader inferences about the target population. Secondly, the time period allocated for the study is relatively short, potentially restricting the ability to capture long-term effects or changes over time adequately. Furthermore, the absence of multicentric trials is recognized as a limitation, as it restricts the inclusion of diverse participant populations from different

geographical locations or settings. Conducting multicentric trials would enhance the study's external validity and increase its applicability to a wider population. Additionally, the study acknowledges the need for more advanced microbiological studies to explore in-depth the complex microbial interactions or phenomena under investigation. By recognizing these limitations, researchers can contribute to the growth and refinement of knowledge in their respective fields and encourage further exploration to address these gaps in future studies.

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