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COMPARATIVE EVALUATION OF SODIUM CHLORIDE RINSE WITH CHLORHEXIDINE RINSE AGAINST ORAL MICROBES IN PATIENTS WITH PERIODONTITIS: A RANDOMIZED CONTROLLED TRIAL

Dental Science

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ABSTRACT

Background: Plaque controlling processes play an important role in the management of periodontitis. Although the most effective method is scaling and root planing (SRP), the mouth rinse methods can be more beneficial as an adjunct. Chlorhexidine (CHX) is used as a standard mouth rinsing agent but despite its efficacy, it poses side effects like teeth staining, recolonization, etc. Due to the side effects of CHX and other chemical plaque controlling methods, there is a constant demand for some alternative solution that can be less harmful, but as effective as CHX. **Methodology:** The randomized trial was registered under the Clinical Trials Registry-India (CTRI Reg no- CTRI/2022/02/040179) on 10th February 2022. A total of 30 periodontitis patients were randomly selected for the study and then divided into two groups. The control group (N=15) was given chlorhexidine and the study group (N=15) was given the salt solution for mouth rinsing for two weeks. Plaque index, gingival index, and real-time PCR analysis were the outcome variables, measured at the baseline and after two weeks of intervention. **Results:** Comparison, within the group, shows that all the values decreased significantly after two weeks of intervention in both the groups. (p-value <0.05) except for the PCR analysis of A. actinomycetemcomitans and T.forsythia. The comparison of variables between the group shows that the baseline data were comparable (p-value > 0.05). After two weeks of intervention, the difference between the two groups was found insignificant which indicates the same efficacy of both the interventions. **Conclusion:** In periodontitis patients, the formation of dental plaques can be delayed and prevented by prescribing the regular mouth rinsing activity to the patients with the help of laboratory standardized salt solution.

KEYWORDS

Periodontitis, Mouthrinse, Plaque control, Saline, Chlorhexidine

INTRODUCTION:

Periodontitis, a multifactorial disease, causes attachment loss and alveolar bone resorption, which may result in tooth loss. It is seen in 15%–20% of middle-aged adults and is considered the alternate most common dental disease worldwide after dental caries. [1] Dental plaque is believed as the main etiopathological factor for periodontal disease which is an aggregate of micro-organisms with their products into the intercellular matrix. [2] The complex microbial plaque biofilms in the subgingival area house over 700 bacterial species and phylotypes. In adults, Treponema denticola, Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Prevotella intermedia, and Tannerella forsythia (formerly Bacteroides forsythias) are linked to the progression of periodontal disease and are considered as strong markers for detection, and prognosis of periodontitis. By detecting the presence of some specific microbial species, an individual's risk status can be assessed.[3-5]

Plaque controlling measures, both mechanical and chemical, play important role in the management of periodontitis. At a professional level, the most effective mechanical method to eliminate dental plaque is scaling and root planing (SRP). However, SRP is effective only in reducing the bacterial load and does not necessarily eradicate all pathogens from deep pockets completely, which may be due to the presence of plaque at inaccessible locations. [6] At the personal care level, the removal of plaque by the normal brushing method alone is also less effective. Chemical plaque control methods like mouth rinses serve as an adjunct to the primary mechanical methods of oral hygiene.

The chemical plaque control methods include the preliminary antimicrobial therapies for the eradication of pocket flora including the use of systemic antibiotics. However, the evidence suggests that such treatment doses might be associated with undesirable side effects, such as drug toxicity, drug interaction, acquired bacterial resistance, and patient compliance. [7,8] In dentistry, mouth rinse activity is a meaningful, cost-effective measure in addition to mechanical and other chemical oral hygiene methods. [9]

The effectiveness of chlorhexidine (CHX) has been well established and is the most commonly used antiseptic agent for mouth rinsing. Despite its high efficacy, it has a few drawbacks e.g. discoloration of teeth, dysgeusia, and risk of recolonization.[10]

The majority of the alcohol-based commercially available mouth rinses also pose the potent side effects such as alteration in taste, an increase of calculus formation, staining of teeth and mucous membranes, and, more rarely, oral mucosa desquamation and parotid swelling when used as a mouth rinse for a prolonged duration. [11] Moreover, while opting for a mouth rinsing agent, affordability is a major point of concern when the Indian population has to be made aware of the daily mouth rinsing activity as an essential measure for maintaining oral health. [12]

Therefore, due to its easy availability, and affordability, the salt solution has been chosen as a mouth rinsing agent in this study to see its effects on bacterial growth and plaque formation. Thus, the focus of this study was to assess the effectiveness of salt (NaCl) water (laboratory graded) rinse in reducing periodontitis-causing microbes. Chlorhexidine mouth rinse has been used as a benchmark control for comparison. The efficacy of the treatment has been examined by the subgingival biofilm analysis with the help of Polymerase Chain Reaction (PCR). Due to the presence of a wide variety of bacterial profiles, PCR has become a more effective tool for the rapid detection of bacterial pathogens with higher sensitivity and specificity as compared to cultivation studies.[13]

MATERIALS AND METHODS:

Study design: A randomized control trial.

The study followed the CONSORT guidelines. **(Figure 1)**

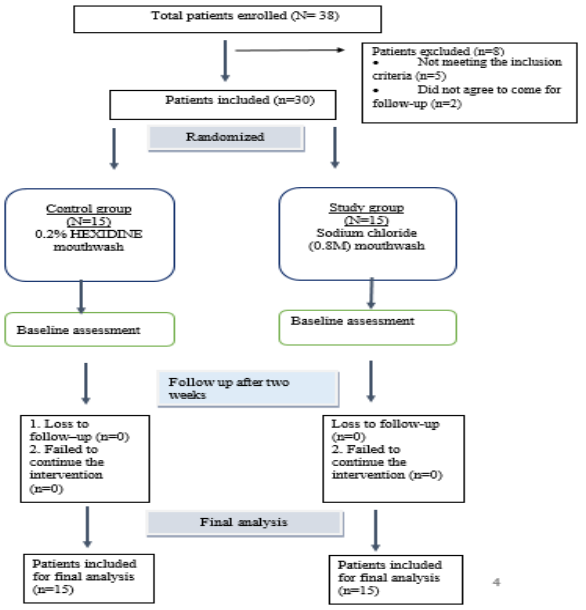

Figure No. 1: CONSORT Guidelines

This study was approved by the human subject's ethics board of the Institutional Ethical Committee of the Army College of Dental Sciences, Secunderabad, and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. and the randomized trial was registered under the Clinical Trials Registry-India (CTRI Reg no- CTRI/2022/02/040179).

Patient selection:
38 patients were assessed for eligibility out of which 30 patients meeting the inclusion criteria with generalized moderate to severe periodontitis (19 males, 11 females; age range 29–54 years;) were selected from the outpatient department (OPD), Department of Periodontology, Army College of Dental Sciences, Secunderabad from February 2022 to April 2022. Written informed consent was taken from all participants recruited in the study. Keeping standard values for alpha error and beta error, the sample size was determined to be 30 which was calculated using G Power software. The study participants were randomly divided into two groups, with 15 participants in each group. The first group which was provided 0.8M NaCl solution for mouth rinsing, was referred to as the study group and the second group which was provided commercially available 0.2% chlorhexidine mouth rinse (HEXIDINE, ICPA HEALTH PRODUCTS LTD), was referred to as the control group.

Inclusion criteria:
Patients with the following criteria were included:
(i) Eighteen years or older and in good general health;
(ii) had more than 20 natural teeth;
(iii) had bilateral maxillary first molars that still had both mesial and distal neighboring teeth;
(iv) had more than 5-mm deep periodontal pockets and bleeding on probing.

Exclusion criteria:
(i) Patients with a history of systemic disease.
(ii) Patients who had undergone oral prophylaxis or any kind of treatment within 6 months.
(iii) Patients with a history of antibiotics usage within 1 week
(iv) Patients using any chemical antiplaque agents within 1 week
(v) Patients wearing any RPD or FPD or orthodontic appliances

Methodology and Sample collection:
The case history was taken and patients diagnosed with periodontitis were selected for the study. The plaque index (Silness and Loe, 1964), and Gingival index (Loe and Silness, 1963) were recorded and baseline subgingival plaque samples were obtained from all the participants for microbes PCR analysis by the clinician, who was blinded to the groups allocated to the individuals.

During the intervention, all the participant's oral hygiene standards were matched. Scaling and root planing were done on all the participants. 0.8 M of sodium chloride is used in the present study which was prepared by dissolving 14.1 g of sodium chloride in 300 milliliters of distilled water in a sterile beaker in the Department of Pathology and Microbiology, Army College of Dental Sciences. 100 milliliters of mouthwashes were dispensed in similar sterile opaque bottles and marked with only the subject number by the study coordinator. Each patient was given three bottles containing 0.8M sodium chloride and 0.2% chlorhexidine mouthwashes in the study and control group respectively by the clinical examiner who was blinded to the contents of the bottles. All participants were instructed to use 10 ml of assigned mouth rinse twice daily for 30 secs between 9:00 to 9:30 am and 9:30 to 10:00 pm for 2 weeks.[14,15] Participants were also instructed to abstain from any other forms of oral hygiene aids, including dental floss during the study period. The plaque and gingival indices were taken and collected on the 2nd week after the beginning of the intervention and again assessed for PCR analysis of periodontopathogens.

Plaque sampling:
After identification of the tooth having a periodontal pocket, gentle air-drying was done. The supragingival plaque was removed carefully with curettes and subgingival samples were obtained by placing a sterile Gracey curette from the apical extent of the deep periodontal pocket (PD ≥5 mm) or gingival crevice and drawing it coronally with slight pressure and transferred to sterile microcentrifuge tubes containing 60 microliters of phosphate buffer saline and stored at -20-degree C and then processed for extracting RNA samples. Those samples were further proceeded to form complementary DNA (cDNA) by reverse transcription for subsequent DNA extraction and real-time polymerase chain reaction (RTPCR) analysis.

The period onto pathogens, assessed by PCR analysis, were the following: P. gingivalis, T. denticola, T. forsythia, and A. actino mycetemcomitans. To follow the RTPCR protocol species-specific primers (Sigma-Aldrich Pvt. Ltd.) were used for different microbes.

Statistical analysis:
The data was gathered and entered into a Microsoft Excel spreadsheet. An Independent t-test of significance was used for intergroup analysis and comparison of the efficacy of the two types of mouthwash on plaque index, gingival index, and microbial PCR analysis at baseline and after two weeks.

Paired t-test was used for intragroup analysis and comparison of the effect/efficacy of the two types of mouthwash on plaque and gingival index and microbial PCR analysis at baseline and after two weeks. Data analysis was carried out using Descriptive statistics and other relevant tests of significance. The p-value was set at 0.05 to be significant and a p-value less than 0.01 was considered highly significant. The confidence level was set at 95% and the power of the study was fixed at 80%. Data were analyzed using SPSS version 26.

OBSERVATION AND RESULTS:
Thirty patients with generalized moderate to severe chronic periodontitis were included in the study. 15 (9 males and 6 females) of them were given Chlorohexidine (CHX) and the rest of the 15 (10 males and 5 females) were given saline solution for the mouth rinsing. In the control group, 45.53± 6.63 years was the mean age of the participants which was comparable with the study group participants i.e. 45.93 ± 8.00 years(**Table No.1).**

Table No. 1. Demographic profile of both the groups.

	Control / CHX group		Study / Saline group	
Age in years (Mean ± SD)	Males (n = 9)	Females (n = 6)	Males (n = 10)	Females (n = 5)
	47.77 ± 5.7	42.16 ± 6.9	41.8 ± 7.58	48.2 ± 7.79
	45.53± 6.63		45.93 ± 8.00	

Table no.2 shows the comparison of the mean values of plaque index and gingival index between the baseline and after two weeks of treatment in the individual group. Both the indices show the fall in their values with both types of mouth rinsing solutions and the difference has been found highly significant in both the groups (p-value < 0.01).

Table No. 2. Intra-group comparison of mean values of Plaque index and Gingival index at the baseline and post-treatment.

Variables	Study group			Control group		
	Baseline	Post-treatment	p-value	Baseline	Post-treatment	p-value
Plaque Index	2.21± 0.37	1.16 ± 0.34	0.0001*	2.21± 0.37	1.2 ± 0.38	0.0001 *
GI index	2.28 ± 0.34	1.29 ± 0.28	0.0001*	2.30 ± 0.36	2.3± 0.36	0.0001 *

After doing the intra-group comparison, the variables were compared between the groups, as shown in **Table No. 3**. At the baseline, the difference in the mean values of both the indices between the groups was found insignificant. After two weeks of treatment, both the variables were decreased in both the groups but the post-treatment mean values were also found insignificantly different when compared between the groups (p-value > 0.05).

Table No. 3. Intergroup comparison of mean values of Plaque index and gingival index.

Variables	Baseline			Post-treatment		
	Study group	Control group	p-value	Study group	Control group	p-value
Plaque Index	2.21± 0.37	2.21± 0.37	1.0	1.16 ± 0.34	1.2 ± 0.38	0.731
GI index	2.28 ± 0.34	2.30 ± 0.36	0.839	1.29 ± 0.28	2.3± 0.36	0.935

Real-time microbial PCR analysis was one of the disease outcomes that had been observed at the baseline and two weeks after the mouth rinsing in both groups**. Table no. 4** shows the difference between the mean values of real-time microbial PCR analysis at the baseline and two weeks post-treatment within the groups. In the study group, with the saline treatment, the PCR analysis shows a reduction in values which is significant in the case of P.gingivalis and T.denticola (p-value < 0.05). Similar findings were observed in the control group with the chlorhexidine treatment.

Table no. 4. The intragroup comparison of mean values of real-time microbial PCR analysis between their baseline and post-treatment values

Micro-organisms	Study group			Control group		
	Baseline	Post-treatment	p-value	Baseline	Post-treatment	p-value
A. actinomycetemcomitans	1.5 ± 0.527	1.08 ± 0.75	0.162	1.00 ± 0.00	1.09 ± 1.21	0.862
P.gingivalis	1.00 ± 0.00	0.44 ± 0.331	0.045*	1.00 ± 0.00	0.41 ± 0.309	0.033*
T.forsythia	1.00 ± 0.00	0.84 ± 0.851	0.733	1.00 ± 0.00	0.867 ± 0.86	0.779
T.denticola	1.00 ± 0.00	0.27 ± 0.183	0.004*	1.00 ± 0.00	0.30 ± 0.137	0.002*

After observing the difference in the PCR values within the groups, the intergroup comparison was done. **Figure No. 2** shows the values of PCR analysis of all assessed microbes after the two weeks of treatment in both groups. The post-treatment values between the groups were comparable as the difference was found non-significant**.** (p-value> 0.05)

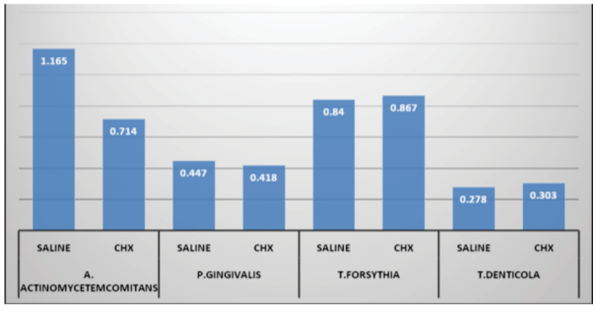

Figure No. 2: Intergroup comparison of post-treatment PCR analysis values.

DISCUSSION:

It was a randomized control trial study to evaluate the efficacy of saline solution in the prevention of plaque formation and consequently the reduction of periodontitis-causing microbes' population. For comparison, one group of periodontitis patients was given salt solution (0.8 M) and another group was given chlorhexidine (0.2%). The Outcome variables were plaque index, gingival index, and real-time PCR analysis to assess the efficacy of the intervention.

At the baseline, all the variables were assessed and both the groups were found comparable with each other. After scaling and root planing, participants of the control and study group were given chlorhexidine and saline solution respectively for rinsing twice daily. After two weeks, all the variables were assessed again and compared. In both the groups individually, the plaque index and gingival index were decreased significantly along with a significant decrease in real-time PCR data in the case of P.gingivalis and T.denticola. Post-treatment, inter-group comparison of all variables shows that the efficacy of saline solution is similar to chlorhexidine.

The findings of the present study have been found in agreement with the previous studies. Aravinth V et.al, compared the salt water rinse with the chlorhexidine mouth rinse and found that Salt water was as effective as chlorhexidine in reducing dental plaque (p = 0.19) and A. actinomycetemcomitans (p = 0.35) count whereas chlorhexidine was superior against S. mutans (p = 0.001), L. acidophilus (p = 0.001) and P. gingivalis (p =0.001). Another study conducted by Rupesh et.al, showed a statistically significant reduction in salivary S. mutans count after 21 days of saturated saline rinsing. They compared and evaluated the effects of saturated saline rinse and 0.02 M alum mouth rinse on salivary S. Mutans levels in children. Gupta et al. in which they evaluated the effect of Aloe Vera mouthwash with chlorhexidine and saline as the placebo on dental plaque. They found that the saline rinse was not as effective as Aloe-Vera and chlorhexidine. The probable reason for not finding the effectiveness of saline mouth rinse was that the concentration of saline in molarity has not been quantitatively measured in this study. There are other limited studies assessing the effect of salt water against L. acidophilus, A. actinomycetemcomitans, and P. Gingivalis.[16-18]

In many studies, herbal agents have been tested and proven to be effective as a chemical mouth wash e.g. neem, tea shallot extract, garlic, aloe Vera, white pepper extract, turmeric, guava leaf extract, and Triphala.[19-22] However, the preparation of herbal mouthwashes requires an intense laboratory approach. Collins JR et al compared the anti-inflammatory efficacy of sodium chloride- and 0.12% chlorhexidine mouth rinses in patients undergoing minimal invasive periodontal surgery. Gingival Index (GI) was evaluated in the whole mouth and the surgical site at baseline (T1), a week later (T2), and 12 weeks (T3) after the treatment. the average GI values showed significant differences between baseline and T2 (p = 0.0005) and baseline and T3 (p = 0.003) in the test group and concluded the sodium chloride-mouth rinse use after periodontal surgery seems to have similar anti-inflammatory properties as CHX mouth rinse and can be used regularly postoperatively after periodontal surgical procedures. The use of saline mouthwash showed an anti-inflammatory effect similar to CHX 0.12% after minimal invasive periodontal surgery which is also seen in the present study. [23] However, in the healing process, its effectiveness has generated controversy, since CHX has been associated with tissue necrosis, inflammatory reactions, and inhibition of regeneration. [24,25]

da Costa et al concluded that using CHX as an adjunct to SRP therapy in chronic periodontitis patients may cause potential tooth staining with less effect on clinical attachment loss (CAL). On the other hand, Saltwater mouthwash is more accessible to the world population, more economic, and can contribute to a significant reduction in plaque formation by its bacteriostatic action.[26]

CONCLUSION:

Dental plaque formation occurs *de novo* in the oral cavity. Elimination of dental plaque completely is practically not possible. Thus, plaque control intends to prevent the growth and maturation of dental plaque microorganisms. In this regard, salt being bacteriostatic can be considered as effective as chlorhexidine in plaque control which is also reflected in the sequel of the present study.

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Conflict of Interests:
No conflict of interest.

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