

Evaluation and Comparison of Antimicrobial Effects of Chlorhexidine (CHX) and Chitosan (CHT) Mouthwash in Chronic Periodontitis (CGP) Patients – A Clinicomicrobiological Study

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Abstract

Aim: The objective of study is to investigate the in vivo antibacterial effects of 0.12% CHX and 2% CHT in adjunct to Scaling and Root Planing (SRP) on plaque microbiota in patients with CGP.

Methods: The study was conducted by collecting supragingival plaque samples from 40 patients with CGP who reported at CDSRC, Ahmedabad. Subjects between 35 and 55 years were randomly allocated to one of the two groups: Group A (n = 20) in which patients were given 0.12% CHX and Group B (n = 20) in which the patients were given 2% CHT after a thorough SRP. Plaque samples were inoculated on nutrient agar and the CFU were counted. The patients were then instructed to use the mouthwashes for one week, after which plaque samples were again collected, inoculated and CFUs were counted.

Results: The mean CFU count reduction after using 0.12% CHX and 2% CHT for one week were 3.563×10^2 & 3.714×10^2 respectively.

Conclusion: Both the mouthwashes were effective in reducing the total bacterial count after one week. We conclude that further investigations are needed to evaluate the potential value of CHT as an effective anti-plaque mouth rinse.

Keywords: Chitosan, Mouthwash, Periodontitis, CFU count, Chlorhexidine.

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I. Introduction

Dental plaque is the primary etiologic factor of periodontal diseases. According to the current concept regarding the etiology of periodontal diseases, three factors determine the risk of active periodontal disease: a susceptible host, presence of pathogenic species, and absence of so-called "beneficial bacteria". The removal of bacterial biofilms from tooth surfaces and plaque control are the main goals of the prevention and treatment of periodontal diseases.^{1,4}

In addition to mechanical plaque control, the application of antiseptic compounds using mouth rinse formulations can complement or replace mechanical removal. Studies showed that many of these antimicrobial agents have inhibitory effects on plaque and gingivitis compared to negative controls or a placebo, in the absence of tooth brushing.^{2,3}

Bacteria colonizes the oral cavity within a few hours after birth. Colonization of the gingival crevice occurs initially by bacterial interactions with the tooth and later by interbacterial interactions leading to the formation of an organized symbiotic community, called biofilm. Recent researches and studies have proved that gingivitis and periodontitis are polymicrobial infections caused by the biofilm-associated bacteria.⁴

Periodontal disease can be eliminated or maintained by using combination of mechanical and chemical plaque control modalities. The antimicrobial oral rinses help in plaque control by reducing the amount of oral micro flora. It is difficult to remove all bacteria by mechanical plaque control; thus, antibacterial mouthwashes can be useful as adjunct. In the current study, the antibacterial effects of two mouthwashes were compared in vivo.⁵

CHX (Chlorhexidine) a cationic bisbiguanide is effective in reduction of several dental plaques microorganisms including *Streptococcus mutans*.⁵ The mechanism of action includes, Substantivity which involves a reservoir of CHX, slowly dissolving from all oral surfaces, resulting in 'Bacterial mileu' in the oral cavity; interactions with external cell components and the cytoplasmic membrane, inducing the leakage of intracellular components, and interactions with cytoplasmic constituents.⁶ CHX has certain side effects like brownish discoloration of the teeth, erosion of oral mucosa, and bitter taste. Therefore the search for new and alternative antimicrobial substances with fewer side effect continues.⁷

Chitosan is made by partial deacetylation product of chitin. It is a widely distributed polycationic biopolymer exhibiting various promising biological activities. These include antimicrobial and antifungal activities next to bio gradable and biocompatible properties.⁸ The precise antibacterial mechanism of chitosan is still unknown but several mechanisms have been proposed. The currently accepted theory states, Chitosan possesses reactive positively charged amino groups that interact with the negatively charged bacterial cell membranes resulting in the leakage of proteinaceous and other intracellular constituents and alteration of cell permeability.⁹

An important property of Chitosan (CHT) is the bioadhesive nature this gives chitosan an ability of good retention on oral surfaces and thus it is suitable to act as vehicle for the release of various oral therapeutical agents.^{10, 11, 12} In certain studies the effectiveness of Chitosan have be studied using specific Plaque biofilm bacteria like *Streptococcus sanguinis* which is one of the major human plaque forming strain with cariogenic potential and was therefore chosen as indicator strain for antiplaque effects of chitosan.¹³

The objective of this study was to compare the antimicrobial effects of 0.12% CHX and 2% CHT on aerobic and facultative bacteria gathered from supragingival plaques of patients with periodontitis.

II. Materials And Methods:-

The study was conducted in the Dept. of Periodontology and Implantology, at the College of Dental Sciences and Research Centre (CDSRC), Ahmedabad, Gujarat in the month of January and February 2017. Sample processing and all other laboratory procedures were done at the Dept. of Clinical Microbiology at the College of Dental Sciences and Research Centre, Ahmedabad, Gujarat.

Sources and Samples: The participants in this study were recruited from patients with Chronic Generalised Periodontitis seeking periodontal treatment at the College of Dental Sciences and Research Centre, Department of Periodontology and Implantology, Ahmedabad. They signed an informed consent form provided by the College.

Inclusion Criteria for the Study were based on the following conditions:

- Erythematous gingiva
- Bleeding on probing
- Age between 35-55 years old
- Periodontal Pockets with > 30% of teeth with depth \geq 4 mm (Figure 1)



Figure 1: Evaluation of Periodontal Pocket Depth.

Exclusion criteria for the Study were based on the following conditions:

- Smoking
- Systemic Diseases
- Pregnancy and Lactation
- Orthodontic or Prosthodontic appliances

- Antibiotic therapy within the last three months
- Allergy to the mouthwash

The Group A mouthwash CHX 0.12% was a commercially available mouthwash HEXIDINE®-EP with composition Chlorhexidine Gluconate 0.12% w/v.

The Group B mouthwash CHT 2% was prepared by mixing 2gm of chitin with 100 ml of 1% acetic acid and mixed for 5 hours in a magnetic stirrer. The prepared Chitosan solution was then transferred in to 50ml bottles, far easy delivery and use by the patients.(Figure 2)



Figure 2: Process of making 2% Chitosan using magnetic stirrer

Instruments for gathering data and validation

Supragingival samples were collected with a sterile curette with the aid of a mouth mirror and cotton rolls. A sterile tube was used for transferring the samples to the laboratory.

Procedure for gathering data

The plaque samples were collected on the first day of visit prior to the oral prophylaxis. For standardization, Oral B toothbrush and toothpaste were given to all patients and they were instructed to brush their teeth by the modified Bass technique three times in a day and use mouthwashes twice a day according to the manufacturer's instructions. The samples were gathered before and one week after using the mouthwashes.

The 40 patients were randomly divided into two groups (twenty patients in each group) using Group A (CHX) and Group B (CHT). Each group of patients used the mouthwashes every 12 hours as follows:

CHX group A: 30 seconds mouth rinsing with 15 ml of CHX mouthwash.³⁰

CHT group B: 30 seconds mouth rinsing with 20 ml of CHT in 10 ml of water.²⁷

Sample Collection

A supragingival plaque sample equal to 1 mg was collected with a sterile curette(Figure 3) and directly immersed in a sterile vial containing 0.9% Normal Saline. To disperse the bacterial cells the vial was agitated and the solution was then immediately sent to the Dept. of Clinical Microbiology to be transferred on to Nutrient Agar Plates.



Figure 3: Sample Collection

Preparation of Nutrient agar plates and inoculation of bacteria

For bacterial colony counting, 0.1 ml of diluted samples was transferred into empty petri plates. Nutrient Agar plates were prepared and sterilised before the samples were inoculated on the plates. The plates were incubated for 24 hours and the colony forming units (CFU) were counted using grid of 2x2 cm any colony with diameter of 1mm were only considered for counting(Figure 4). The same method of sample collection and inoculation was repeated after one week when the subjects completed the one week regimen of mouthwash use.

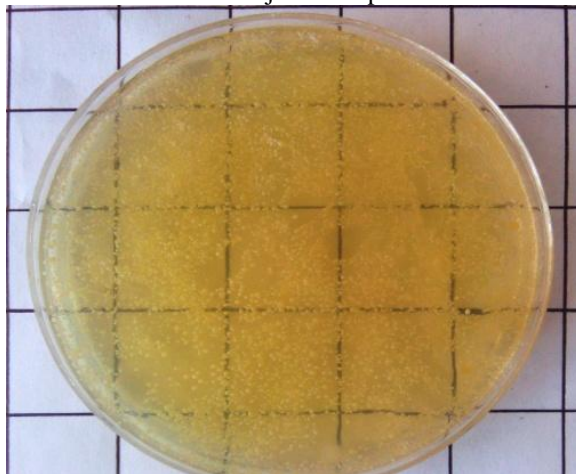


Figure 4: 2x2cm Grid used for the colony counting.

III. Data Analysis

Collected data were studied by coding the variables in Excel format and Paired Student t-test was used to compare the antimicrobial effect of the two mouthwashes. P-values less than 0.05 were considered statistically significant.

IV. Results

Tables 1 summarizes the bacterial counts before and after one week of use of 0.12% CHX and 2% CHT mouthwashes in patients with Chronic Generalised Periodontitis with the help of Student t-test.

Group	Mouthwashes	CFU	Mean(x10 ³)	Standard Deviation
Group A	CHX	Before	5.471	0.774
		After	1.908	0.319
Group B	CHT	Before	5.593	0.635
		After	1.879	0.392

Table 1: Bacterial colony counts (CFU) pre and post treatment with two mouthwashes CHX and CHT

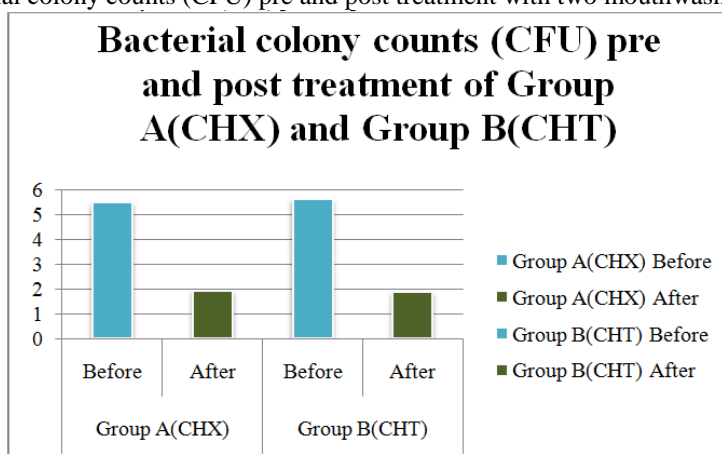


Figure 5: Bacterial count reduction after one week use of Group A (0.12% CHX) and Group B (2% CHT)

Table 1 shows the relationship of bacterial CFU count pre-treatment and one week after the use of Mouthwash of Group A (CHX) and Group B (CHT). Both the mouthwashes were effective in reducing bacterial CFU counts. Figure 5 shows the relationship between the two groups the pre-treatment CFU count were almost

similar in the two groups and even after one week follow up; the bacterial count reduction was significant but the difference in value between Group A and Group B was not that significant.

Table 2 shows the relationship between mean reduction in bacterial count of both Group A (CHX) and Group B (CHT).

Table 2: The mean bacterial count reduction between Group A and Group B

	Mean CFU reduction(x10 ²)	Standard Deviation	P-value
Group A	3.563*	0.756	≤ 0.05
Group B	3.714*	0.706	≤ 0.05

*The mean difference is significant level at p<0.05

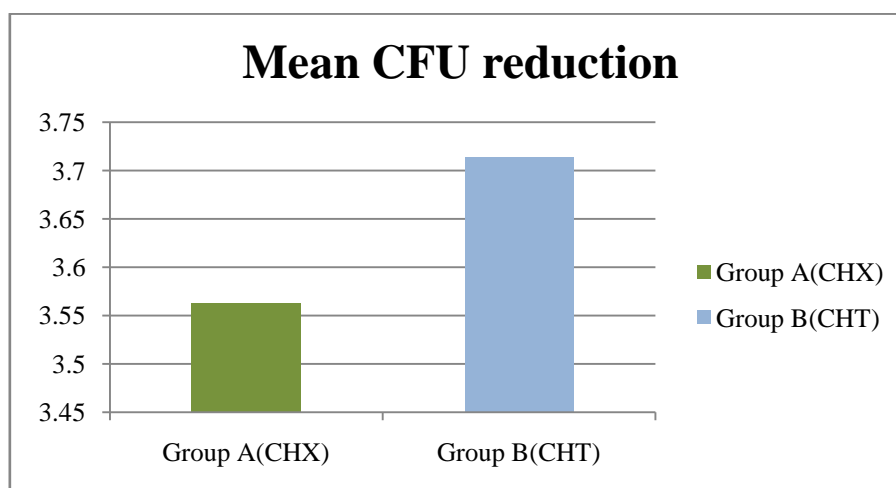


Figure 6: Mean bacterial count reduction after one week use of Group A (0.12% CHX) and Group B (2% CHT)

Figure 6 shows that both the mouthwashes are effective in reducing Bacterial count but Group B (CHT) was slightly more effective in reducing in more bacterial colonies in one week as compared to that of Group A (CHX).

V. Discussion

The aim of this study was to evaluate and compare the antimicrobial effect of two mouthwashes 0.12% CHX and the 2% CHT in patients with Chronic Generalised Periodontitis over a period of one week follow up. Results of this study indicated that the mouth rinses we evaluated demonstrated effective antibacterial effect on bacteria in the oral cavity. These conclusions were supported by the clinical parameters and microbiologic outcomes.

In the present study, when comparing the mean reduction of bacterial CFU's the highest value obtained was 3.714 x 10² which were by Group B (2% CHT) mouthwash over a period of one week in patients with Chronic Generalised Periodontitis as an adjunct to Scaling and Root Planing. Although the difference in the mean bacterial count reduction of Group A and Group B is not that significant. This observation might be explained by the short experimental period and small sample size.

Several in vitro and in vivo studies have proved the long-term efficacy of CHX mouth rinses^{14,15,16} and 0.2% CHX has been accepted as the gold standard for its bacteriostatic action for 8-12 hours.¹⁹ In order to reduce side effects of CHX, the use of CHX mouth rinses of lower concentrations was considered, and decreased side effects were reported.^{17,18} As for plaque inhibition, no differences were observed between 0.1%, 0.12%, and 0.2% CHX mouth rinses.^{20,21} However, it has been suggested a study that evaluated 0.1% CHX mouth rinse, that the degree of CHX inactivation was caused by its formulation.²²

Concentration of 0.12% CHX appeared to be as effective as 0.2%, if the volume of the rinse was increased to 15 mL.²³ The optimal dose of CHX is generally considered to be a regime of 20 mg twice a day,^{24,25} which balances efficacy against local side effects and also improves user acceptability.

The incorporation of chitosan in products, particularly oral care products, has not been extensively explored. While there are several antimicrobials/antibiotics that may possess higher activity or require lower concentrations than chitosan to be active against oral pathogens and their biofilms, but neither possesses the biocompatibility nor the other inherent bioadhesiveness property of chitosan.

Currently there are no reports of any known antimicrobial resistances to chitosan. The antimicrobial action and bio-adhesiveness of Chitosan (CHT) appears to be a perfect match for usage in oral care products however, most mouthwashes used nowadays are carefully crafted formulations with numerous compounds

working together to obtain antimicrobial activity, among other properties.²⁶ In a pilot study to evaluate the taste of chitosan mouthwash without any additional flavouring agent, the taste was found to be unacceptable and hence a dilution of 10mL was used in this study to facilitate patient compliance.

While the inclusion of chitosan in these formulations would be possible, due to chitosan's high reactivity, that makes it a highly sought polymer, would be a double edged sword as its chemical interactions would create havoc and destabilize most formulations. Thus the best route for chitosan incorporation into oral care seems to be the development of novel products drawn specifically to potentiate Chitosan's (CHT) properties.²⁶

Overall the results of the current study validate a stable and active chitosan based mouthwash formulation for human usage. The chitosan mouthwash possesses lower toxicity and higher antimicrobial activity than the commercial mouthwash tested. Hence chitosan mouthwash is a safer, valid and viable alternative to the already existing mouthwashes.²⁷

There have been a few studies reporting the effects of CHT and CHX combinations on oral microorganisms. **Giunchedi et al. (2002)**²⁸ evaluated CHX buccal tablets prepared using drug loaded CH microspheres. Combining CHT microspheres as controlled drug delivery systems with CHX not only prolonged the release of the drug in the oral cavity but also improved the antimicrobial activity of CHX.

In a study, **Decker et al. (2005)**²⁹ evaluated CHX on plaque combination to improve antiplaque strategies. In that study, CHX (0.1%) was used as the positive control, saline was the negative control, and two CHT derivatives together with their CHX combination were attached to *Streptococcus sanguis* for 2 minutes. In their results, the CHX & CHT combination was stronger than CHX alone, because it united the bioadhesive properties of CHT with the antibacterial activity of CHX which resulted synergistically in a superior antiplaque effect to CHX alone.

VI. Conclusion

The results revealed that both the mouthwashes are effective in maintaining low bacterial counts in the mouth. However, since the beneficial role of the presence of commensal species in the oral cavity has been established, the need to maintain constantly low bacterial counts in the mouth is still under debate. The results obtained from this study revealed that both the mouthwashes might decrease the number of bacteria in the oral cavity effectively.

This one week randomized clinical trial demonstrated that both the mouthwashes had comparable antimicrobial activities for patients with Chronic Generalised Periodontitis. The promising outcomes of this study could allow CHT to be considered as a mouth rinse. The precise antibacterial mechanism of CHT is still unknown. It is concluded that further investigations are needed to evaluate the potential value of CHT as an effective antiplaque mouth rinse.

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