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ORIGINAL RESEARCH


# Clinicomicrobiological Evaluation of 2% Chitosan Mouthwashes on Dental Plaque

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## ABSTRACT

**Aim:** This study was conducted to evaluate microbiological and clinical effects of a chitosan chlorhexidine (CH) mouthrinse on plaque control.

**Materials and methods:** Subjects were divided into three groups. Group I included 15 subjects who used 0.2% chlorhexidine digluconate (CHX), group II included 15 subjects who used 2% chitosan (CH) solution, and group III involves 15 subjects who used 0.2% chlorhexidine/2% CH combination. Plaque index (PI), gingival index (GI), and probing depth (PD) were recorded at the baseline, on day 0, and after 4 days. Supragingival plaque samples were subjected for microbiological evaluation. Statistical analysis was done using statistical software IBM Statistical Package for the Social Sciences (SPSS), version 21.

**Results:** Plaque index was lowest in group I at day 0, while it was highest in group III. At day 4, PI was highest in group II, while lowest in group III. Gingival index was lowest in group I and highest in group II at day 0, and lowest in group I and highest in group III at day 4. There was no statistical difference in *Streptococcus mutans (S. mutans)* count between groups at any time interval.

**Conclusion:** Both chitosan and CH were found to be effective in controlling plaque. However, a combination of both provides even better results.

**Clinical significance:** The present study showed that chitosan can be used as an antiplaque agent.

**Keywords:** Chitosan, Chlorhexidine, Plaque.

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## INTRODUCTION

Dental plaque is one of the important factors leading to periodontal diseases. The prevention and treatment of periodontal diseases involve removal of plaque and bacterial biofilms from tooth surfaces. For the better management, mechanical as well as chemical plaque control is required. Mechanical plaque control involves the use of toothbrushes, whereas chemical plaque control includes various antiseptic mouthwashes. These antimicrobial (chemical) agents have inhibitory effects on plaque and gingivitis.[1]

Phase I therapy for prevention of periodontitis includes the use of antimicrobial agents. Recently, a variety of antimicrobial agents have been tested that prevent gingivitis as well as periodontitis. Bisbiguanides chlorhexidine (CH), sanguinarine, metal salts, essential oils, phenols, and fluorides are common microbial agents.[2]

The CHX is among various antiplaque agents which possesses bactericidal and bacteriostatic activities. Its ability in prevention of disease is well documented. The 0.2% CHX has very low toxicity, strong affinity for epithelial tissues, and mucous membranes. Apart from its

beneficial effects, it has harmful effects, such as brown staining of the teeth and tongue, altered taste, increased supragingival calculus deposition, and rarely painful desquamations of the oral mucosa.[3]

Chitosan CH, a natural polysaccharide, is a chemical agent that helps in prevention of plaque formation on teeth. It possesses antimicrobial activity. It has an additional benefit of enhanced retention on the oral mucosa. It is better than CHX in terms of nontoxicity, biocompatibility, and biodegradability. Low-molecular-weight chitosan prevents the adsorption of *S. mutans* onto hydroxyapatite crystal of teeth. It has an antibacterial effect on *S. mutans*, *Aggregatibacter actinomycetemcomitans*, and *Porphyromonas gingivalis*. The CH, both CHX and CH in combination, has a synergistic antiplaque effect.[4]

This study was conducted to determine the microbiological and clinical effects of chitosan CH mouthrinse on plaque inhibition.

## MATERIALS AND METHODS

This study was conducted in the Department of Periodontology in the year 2015. It included 45 periodontally healthy subjects of both gender. Written informed consent was obtained from participating subjects. Ethical clearance was taken from the institutional ethical committee. Following inclusion criteria was used—subjects without anti-inflammatory drugs use, subjects with no history of antibiotic in the past 6 months, and subjects not using tobacco products. Exclusion criteria included subjects allergic to CHX or CH derivatives, subjects with fixed or removable prostheses or orthodontic appliances.

After thorough oral prophylaxes, all the participants were divided into three groups. Group I included 15 subjects who used 0.2% CHX, group II included 15 subjects who used 2% CH solution, and group III involves 15 subjects who used 0.2% CHX/2% CH combination.

The PI, GI, and PD were recorded at baseline, on day 0, and after 4 days. All clinical parameters were measured with a GoldmaneFox Williams probe calibrated in millimeters at six sites per tooth (mesio-, mid-, and disto-buccal and mesio-, mid-, and disto-palatal). Supragingival plaque samples for microbiological sampling were obtained from 14, 24, 34, and 44: mesiobuccal on day 0; distobuccal on day 1; mesiopalatal on day 2; and distopalatal surfaces on day 4. Results were tabulated and subjected to statistical analysis using statistical software IBM SPSS, version 21 and analysis of variance test; $p < 0.05$ was considered statistically significant.

## RESULTS

Table 1 shows mouthrinse groups: Group I—0.2% CHX, group II—2% CH solution, and group III—0.2% CHX/2% CH combination. Table 2 indicates PI and GI at day 0 and day 4. Plaque index was lowest in group I at day 0, while highest in group III. At day 4, PI was highest in group II, while lowest in group III. The difference was statistically significant ($p < 0.05$). Gingival index was lowest in group I and highest in group II at day 0, and lowest in group I and highest in group III at day 4. The difference was statistically non-significant ($p < 0.05$).

**Table 1:** Division of mouthrinse groups

| *Group* | *Group I* | *Group II* | *Group III* |
|---|---|---|---|
| Solution | 0.2% CHX | 2% CH solution | 0.2% CHX/2% CH combination |
| Number | 15 | 15 | 15 |

**Table 2:** PI and GI in all groups

| *Group* | *Day* | *PI* | *GI* |
|---|---|---|---|
| I | 0 | 0.02 | 0.01 |
| | 4 | 0.45 | 0.41 |
| II | 0 | 0.03 | 0.2 |
| | 4 | 0.62 | 0.51 |
| III | 0 | 0.05 | 0.03 |
| | 4 | 0.36 | 0.52 |

$p < 0.005$

**Table 3:** *S. mutans* and *C. albicans* levels in groups

| *Group* | *Day* | *S. mutans* | *C. albicans* |
|---|---|---|---|
| I | 0 | 246 | 141 |
| | 2 | 220 | 145 |
| | 4 | 240 | 146 |
| II | 0 | 246 | 150 |
| | 2 | 240 | 148 |
| | 4 | 238 | 148 |
| III | 0 | 248 | 149 |
| | 2 | 250 | 147 |
| | 4 | 246 | 146 |

$p < 0.001$

Table 3 shows that *S. mutans* and *Candida albicans (C. albicans)* levels were statistically significant on days 0 to 2 ($p < 0.05$) and days 0 to 4 ($p < 0.001$). Higher *S. mutans* amount was obtained in group I on day 0, in group III on days 0 and 1, and in group II on day 0, whereas no statistical differences were observed in *S. mutans* amounts between groups at any time interval ($p > 0.05$). The lowest *C. albicans* amounts were obtained in group I for all measurements, and group II had a higher *C. albicans* amount on day 0.

## DISCUSSION

Chitosan has broad antibacterial and antifungal characteristics. This property has enhanced its use as disinfectant. Studies have shown that chitosan is more effective in inhibiting the growth of bacteria than are chitosan oligomers.[5] In general, chitosan displays greater antifungal

activity than chitin, but chitosan is less effective against fungi that possess a chitin component in their cell walls. The antibacterial activity of chitosan arises from a combination of both bacteria cell binding and deoxyribonucleic acid binding mechanisms.[6] This study was conducted to determine the microbiological and clinical effects of a chitosan CH mouthrinse on plaque inhibition.

Clinicomicrobial effect of different mouthrinses (2% CHX, Chitosan (CH) and combination of CH with chlorhexidine) was evaluated on 45 subjects using GI and periodontal index. The lowest scores were obtained in group III after 96 hours of the plaque accumulation period (Table 2). This explains that the combination of chitosan and CH provides better antiplaque effect than CHX alone. Group III showed better results than other groups. This is similar to results by Jenkin et al.[7] We found that there was no significant alteration in probing pocket depths in all the groups. This is in agreement with the findings of Beiswenger et al.[8] No significant differences were seen in PIs or GIs after 4 days of a plaque accumulation period when we compared 0.2% CHX with 2% CH. This favors the possibility of using CH as an alternative chemical agent for managing patients who show side-effects associated with CHX.[9]

Van Strydonck et al[10] compared 0.12% CHX with 0.05% cetylpyridinium chloride and 0.2% CHX and found that there was no significant difference in plaque accumulation after 3 days in both groups. Similarly, our results showed better results in group III as compared with other groups. Costa et al[11] concluded that chitosan is effective against most of the microorganisms and they suggested it as an alternative to traditional mouthwashes. Decker et al[5] from their study suggested that combination of chlorhexidine with chitosan is more effective in plaque control. Decker et al[12] and Costa et al[13] stated that the antiplaque effect of chitosan is because of its antiadhesive properties toward microorganisms. Some researcher also stated that chitosan can be used effectively in dentifrices to improve oral hygiene, since it reduces plaque by 70%.[14]

Uraz et al,[15] through a randomized clinical trial, evaluated clinical and microbiological role of chitosan on dental plaque and found decrease in microbiological count (*S. mutans* and *C. albicans* levels) in CH and chitosan groups. Chen and Chung[16] evaluated the antibacterial role of chitosan in an *in vivo* and *in vitro* method at different temperatures (25–37°C) and pH values (pH 5–8). They found significant antibacterial effect of chitosan similar to commercial mouthwashes. They concluded that water-soluble chitosan may be a viable alternative to commercial mouthwashes in the future. Costa et al[13] accessed the potential use of high- and low-molecular-weight chitosans as an oral antimicrobial agent and observed that in a week's time there was little to no decrease in efficiency. They also found that chitosan was capable of inhibiting biofilms formed by two microorganisms and was capable of acting on mature biofilms leading to significant reductions (94%) in biofilm survival. Costa et al[17] evaluated the safety of the chitosan and validated, *in vivo*, the biological activity ascertained *in vitro*. Through Ames, methylthiazol tetrazolium, and V79 chromosomal aberration assay, antimicrobial activity was evaluated. They observed that the chitosan mouthwash was safe, presenting lower cytotoxicity than a commercial mouthwash, and that it effectively reduced viable counts of *Streptococcus* spp. and *Enterococcus* spp. Several studies have shown the antimicrobial effect of chitosan on dental plaque as well as dental caries-producing microbes. Aliasghari et al[18] evaluated the antimicrobial effect of chitosan over nonchitosan product, and they observed its effect on cariogenic bacteria also. Nair et al[19] evaluated the *in vivo* effect of CH and chitosan on plaque microbials and they observed mean colony-forming units count reduction after using 0.12% CHX and 2% chitosan for 1 week and concluded that both are effective and a combination of both the agents is more effective. Venkatesh Babu et al[20] compared CH with cacao bean husk extract mouthrinses for antimicrobial efficiency on 50 children of both sexes in the age group of 6 to 10 years and observed no significant difference in *S. mutans* counts in saliva during follow-up visits. They concluded that cacao bean husk extract mouthrinse can be used as a mouthrinse alternative to CH Bagis et al[21] evaluated staining quality of CH for 3 weeks and found natural staining of CH on teeth.

The present study showed the antimicrobial effect of chitosan against plaque microbiota, and hence, it can be used as an alternative mouthwash. Further long-term clinical studies are required to prove its effect.

## CONCLUSION

Both chitosan and CH are found to be effective in controlling plaque. However, combination of both provides even better results. Chitosan can be used as an alternative mouthwash. This has opened the options in the management of periodontitis.

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