Comparative Evaluation of Chitosan Chlorhexidine Mouthwash in Plaque Control: A Preliminary Randomized Controlled Clinical Trial

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Abstract

Aims: The purpose of the present study was to compare the effect of a new mouth wash formulation consisting of chlorhexidine and chitosan on dental plaque and its reduction to that of chlorhexidine or chitosan alone.

Materials and Methods: This study was a single-blind randomized clinical trial with a parallel group design of 3 months duration. Patients (20-40 years) who fulfilled the inclusion and exclusion criteria were assigned equally to group 1: chlorhexidine (0.2%), group 2: chitosan (0.5%) or group 3: chlorhexidine - chitosan combination group. The clinical parameters were recorded at baseline, 6weeks and at 3months. All patients received thorough oral prophylaxis and were instructed to rinse with 10ml of mouthwash twice daily for 1 minute.

Results: The combination of chitosan and chlorhexidine showed a statistically significant reduction ($p<0.05$) in plaque indices from baseline at all time intervals when compared to that of chlorhexidine or chitosan alone.

Conclusion: Our study demonstrates that by unifying the properties of chitosan and chlorhexidine may result in a superior antiplaque effect than that of chlorhexidine alone.

Keywords. Gingivitis, Chlorhexidine, Chitosan, Antiplaque agents, Mouthwash

Introduction

Bacterial plaque is the most important local factor implicated in the etiology of periodontal diseases, and its removal acts as a decisive aspect in the prevention and treatment of periodontal diseases (Neto *et al*., 2008). The development of dental plaque into a specific form of biofilm can be negatively affected by plaque control (Decker *et al*., 2005).

Good supragingival plaque control has been shown to affect the growth and composition of subgingival plaque, and reduce calculus formation. Plaque control includes the use of mechanical procedures as well as chemical agents which retards plaque formation (Hellstrom *et al*., 1996). In order to improve adequate removal of plaque by mechanical means, there is interest in the use of antimicrobial agents as an adjunct to mechanical approaches (Neto *et al*., 2008; Teles *et al*.,2009)

Chlorhexidine is one of the most effective antimicrobial agents available for plaque control. It is considered as the "gold standard" among antiplaque agents. It is a cationic bisbiguanide with broad antimicrobial activity, low cellular toxicity and a strong affinity for binding to the skin and mucous membranes. Chlorhexidine has a wide spectrum of activity encompassing gram-positive and gram-negative bacteria, yeasts, dermatophytes and some lipophilic viruses.(Jones *et al*., 1997). Chlorhexidine products have been limited due to some distinct

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adverse side-effects associated with it. The main effects includes the varying degrees of brownish staining of teeth, tongue and restorations, loss of taste sensation, oral mucosal erosions and enhanced supragingival calculus formation (Greenstein *et al.*, 1986).

Recently, chitosan has shown various promising biological activities such as antimicrobial, antifungal, biodegradable and biocompatible properties (Decker *et al.*, 2005). Chitosan kills the bacteria by altering its cellular permeability. Another useful property of chitosan is its bioadhesive nature, having the ability of good retention on oral surfaces, so that it exhibits sustained or prolonged retention and action on oral surfaces (Needleman *et al.*, 1997). Chitosan is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (crabs, shrimp, *etc*) and cell walls of fungi .

In dentistry, chitosan has been used as an antiseptic (antibacterial) in various forms such as gels (Bhattarai *et al.*, 2010) and mouthwashes (Vilasan *et al.,* 2013). In the past it has been used as a carrier system for the local delivery of various drugs (Bhattarai *et al.*, 2010) due to its excellent properties such as absorbability, malleability, and cohesive threshold concentration to hold and gradually release drugs with optimal resorption (Bleier *et al.*, 2009). It also demonstrates anti-inflammatory activity by modulating prostaglandin E_2 levels (Pichayakorn and Boonme 2013).

It has been extensively reported in the literature that the combination of chitosan (0.5%) with chlorhexidine (0.2%) in the gel form improves the antimicrobial activity of chlorhexidine with absence of the considerable side effects of chlorhexidine. Therefore, there is a good clinical rationale for the combination of bioadhesive chitosan (0.5%) with chlorhexidine (0.2%) in a mouthwash formulation. In view of this fact, an *in vitro* study was conducted previously by our study group with the aim to evaluate and compare the effect of new mouthwash formulation consisting of chlorhexidine (0.2%) and bioadhesive chitosan (0.5%) on dental plaque bacterial reduction, to that of chlorhexidine or chitosan alone and the CHX + CHT combination showed better results compared to CHX or CHT alone (Vilasan *et al.,* 2013).

To date, there is only one report of a randomized clinical trial comparing the efficacy of a mouthwash composed of chitosan (2%) and chlorhexidine (0.2%) (Mhaske *et al.,* 2018). However, there is a need for more studies to evaluate the effectiveness of chitosan combination with chlorhexidine in mouthrinse formulations. Therefore, the present study is carried out with the aim to evaluate the clinical efficacy of chitosan (0.5%) and chlorhexidine (0.2 %) mouthwash formulation.

Material and methods

Study population

Sixty periodontally healthy participants (30 females and 30 males; with a mean age of 30 ± 0.25 years) were included in the study. The study was conducted by the Department of Periodontology of Krishnadevaraya College of Dental Sciences and Hospital, Bangalore India. Approved by the Ethical Committee Rajiv Gandhi University of Health Sciences, Bangalore, India. After screening for suitability, all participants who fit the criteria volunteered, received verbal and written descriptions of the study design, and signed informed consent forms.

Ethical principles

This was full-mouth, randomized, controlled parallel group design study conducted in a blinded manner approved by the Research Ethics Committee, Krishnadevaraya College of Dental Sciences and Hospital, affiliated to the Rajiv Gandhi University of Health Sciences, Bangalore, India. The study was conducted from April 15th 2019 to June 15th 2019 in Department of Periodontics, Krishnadevaraya College of Dental Sciences and Hospital, Bangalore, in accordance with the Helsinki decalaration of 1975, revised in 2008.

All participants were informed about the objective of the research as well as the risks and benefits of participating in the study. Before the start of the study, the participants signed an informed consent form.

Inclusion and exclusion criteria

Individuals with pre-existing dental plaque and gingivitis with overall mean plaque index score of 1.8 (Turesky Gilmore Glickman modification of the Quigley and Hein index), gingivitis score of 1 (Silness and Löe index), no attachment loss, minimum of 20 natural teeth without artificial crowns were included in the study. Patients allergic to chlorhexidine or chitosan with history of allergic reactions, who have received any periodontal therapy, surgical or non-surgical within the past 6 months of baseline examination, who have had antibiotics within 6 months prior to the study having systemic diseases, who have acute pulpal, periapical or periodontal pathology, pregnant patients, patients using antibacterial mouthwash or medicated toothpaste within six months of baseline data collection, receiving medications such as anti-inflammatory, cardiac, epilepsy, or other medications which could affect periodontal health were excluded from the study.

Preparation of mouthrinse formulation

The chitosan mouth rinse formulation in this study had the following composition (expressed as w/w %) ; 0.5% chitosan (molecular weight of 272 kDa and a degree of

84% deacetylation), 15% ethanol, 10% glycerine, 0.008% sodium saccharine, 1% polyoxyethylene hydrogenated castor oil, and 0.3% flavour in deionized water. CHX at 0.2% was incorporated into the CHT solutions. All study products were blinded. All three mouth rinses were delivered in brown bottles. The bottles were labelled A, B, and C by an investigator: bottle A contained 0.2% CHX, bottle B contained 2% CHT, and bottle C contained the 0.2% CHX/2% CHT combination

Training and calibration

The researchers (MLVP and AV) in charge of implementing the treatment with mouthwashes were trained to standardize the concentration and quantity of application. The researchers (BVK) in charge of making the readings were trained and calibrated as regards intra- and inter-examiner reproducibility. The intraclass correlation coefficient was 0.99.

Randomization, concealment of allocation and masking

Restricted randomization was done by an independent researcher (AD). The allocation was kept secret within opaque, sealed envelopes. The masking of the patients was facilitated by the fact that the mouthwash had a similar colour making it impossible for participants to know which treatment they had received in each quadrant. The operator was also blinded to the mouthwash. Only at the time of use of mouthwash, the envelope was opened.

Interventions

The study protocol is summarized in (Figure 1). Before the experimental phase, each participant received oral professional prophylaxis to remove all plaque, calculus, and stains from the teeth. This was performed using hand instruments and rotating brushes with polishing paste.

Sixty patients were randomly allocated based on the computer generated randomization chart into one of the following 3 groups consisting of 10 patients each. In group 1, 20 patients rinsing with 20 ml of 0.2% chlorhexidine twice daily for 3 months were allocated. In group 2, 10 patients rinsing with 20 ml of 0.5% chitosan twice daily for 3 months were allocated. In group 3,10 patients rinsing with 10 ml of chlorhexidine chitosan combination twice daily for 3 months were allocated. After distribution of respective mouthwashes, patients were instructed to rinse with 10 ml of the solution (without dilution) for one minute after breakfast and dinner and not to eat or drink thereafter for 30 minutes. Mouth rinsing was performed twice a day (after breakfast and in the evening) for 15 days. Participants were provided with a kit containing dental floss, a standard toothbrush, and conventional toothpaste for oral hygiene, and were instructed to use it after every meal until the next visit. Participants were instructed to brush twice daily and rinse twice daily half an hour after brushing. Participants were instructed to use floss after every meal. They were instructed to maintain a dairy and during every recall visits they were instructed to bring dairy and mouthwash bottles. Written instructions explaining how to use the mouth rinses were provided.

Clinical assessment

The clinical examinations were performed by the examiner (BVK) who was blinded under experimental conditions at baseline before treatment (scaling), at 6 weeks and at the end of 3 months after treatment. Data were recorded by plaque index using Turesky Gilmore Glickman modification (1970) of the Quigley and Hein (1962) index, gingival index using Loe and Silness index (Loe and Schiott, 1970) and bleeding on probing using Ainamo and Bay index. The presence or absence of gingival bleeding was determined by gentle probing of the gingival crevice with a periodontal probe. The appearance of the bleeding within 10 seconds indicates a positive score, which was expressed as a percentage of the total number of gingival margins examined.

Statistical analyses

Sample size determination was done before starting the study using G power software. To determine the sample size , a calculation for the comparison of means was used. Calculation was done by keeping the effect size of 0.4 and alpha error of 0.05 with 80% power and an additional 10% to compensate for losses determined that 60 patients were randomly allocated in 3 groups consisting of 20 patients each would be sufficient. The standard deviation was obtained in a similar previous study (Uraz *et al.,* 2012*)*. Data analysis was carried out using Statistical Package for Social Science (SPSS) package. The data collected were entered in Microsoft Excel and Statistical analyses were performed using the Statistical Package for Social Science (SPSS) package. Proportions were compared using Chi-square test of significance. One way analyses of variance were used to test the difference between groups followed by post-hoc least squares difference test (LSD). In all the above test the "p" value of less than 0.05 was accepted as statistically significant.

Results

A total of 60 patients were evaluated. The parameters were recorded at baseline, 6 weeks and 3 months. The parameters included in the study were Plaque score (PI) and Gingival score (GI). The descriptive baseline characteristics of the 3 groups are depicted in (Table 1). There were no records of adverse reaction to any

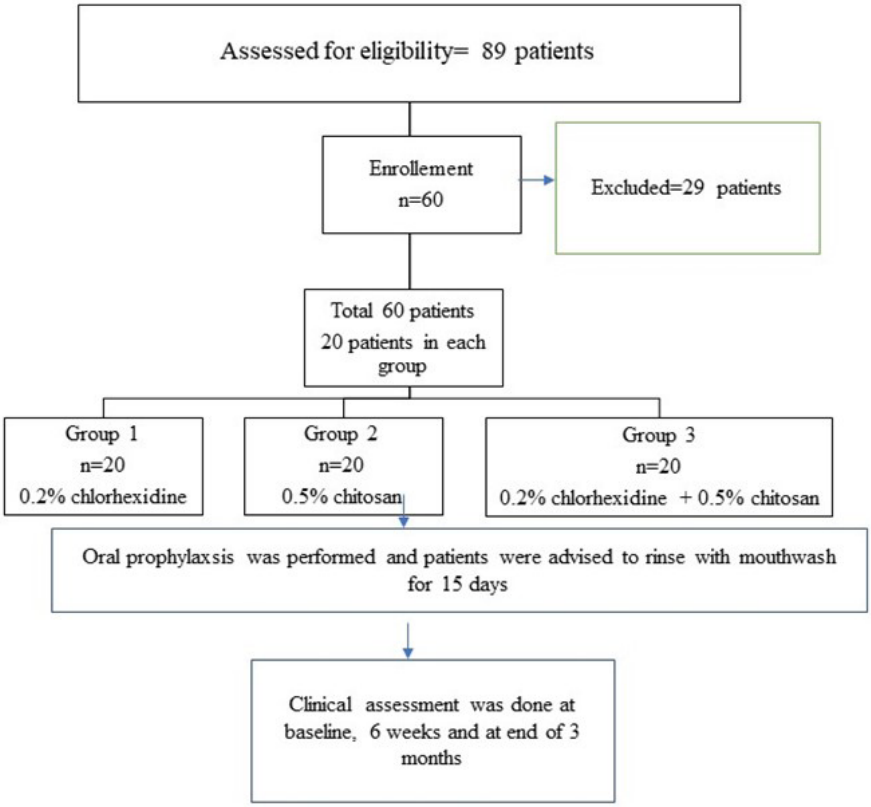

Figure 1. Randomization chart

Table 1. Demographic analysis of the study groups using different mouthrinses

	0.2% CHX	**0.5% CHT**	**0.2% CHX + 0.5% CHT**	***p* value***
Total number of participants	20	20	20	
Age(mean 30 ± 0.25 years) (range 20-40 years)	31.17 ± 2.45	30.72 ± 2.08	29.13 ± 2.39	0.76
Number of male/female subjects	10/9	11/10	9/11	0.43

*Chi-square test

of the mouthrinses used. Baseline scores for all three groups were not significantly different from each other. The site activity score after treatment of all 3 groups were significantly lower than before treatment ($p<0.05$). Analysis of variance (ANOVA) was used to analyse the reduction in plaque and gingivitis in the three groups. The site groups activity score after treatment of chlorhexidine and chitosan group was significantly lower than those of chlorhexidine and chitosan group ($p<0.05$) alone (Table 2).

There was a progressive decline in the plaque and gingivitis scores at the 5% level of significance (Figures 2 and 3). The chlorhexidine + chitosan group showed remarkably greater reduction as compared to the chlorhexidine and chitosan group alone and it was statistically significant. Multiple comparisons were obtained by post-hoc LSD test. The difference in the decrease in plaque ($p< 0.00$ at 6 weeks and $p< 0.00$ at 3 months) and gingivitis ($p <0.00$ for 6 weeks and $p <0.00$ for 3 months) between the chlorhexidine, chitosan and chlorhexidine + chitosan group was statistically significant . To summarize our data revealed significant difference between chlorhexidine, chitosan and chlorhexidine + chitosan for any clinical parameters throughout the study. There were no adverse effects seen in the patients.

Table 2. ANOVA and post hoc LSD for the three groups

Baseline comparison	**Multiple comparison (p value)**							
	0.2% CHX group (a)	**0.5% CHT group (b)**	**0.2%CHX+ 0.5%CHT (c)**	**F value (ANOVA)**	***p* value**	**(a-b)(not in %)**	**(b-c)**	**(a-c)**
Plaque index	3.19 ± 0.18	2.87 ± 0.19	3.76 ± 0.20	56.92	0.00	0.31	-0.89	-0.58
Gingival index	1.73 ± 0.18	1.80 ± 0.11	1.10 ± 0.03	11.84	0.00	-0.06	-0.20	-0.26
6 week comparison								
Plaque index	2.60 ± 0.20	2.62 ± 0.21	2.13 ± 0.33	12.14	0.00	-0.03	0.50	0.47
Gingival index	1.50 ± 0.12	1.63 ± 0.10	1.27 ± 0.04	39.978	<0.00	-0.12	0.36	0.23
3 months								
Plaque index	1.87 ± 0.21	1.99 ± 0.16	1.26 ± 0.27	32.30	0.00	-0.12	0.73	0.61
Gingival index	1.30 ± 0.09	1.50 ± 0.10	1.04 ± 0.03	85.48	< 0.00	-0.20	0.46	0.26

Results are expressed as mean ± standard deviation. # Multiple comparison by post hoc LSD, *$p < .05$

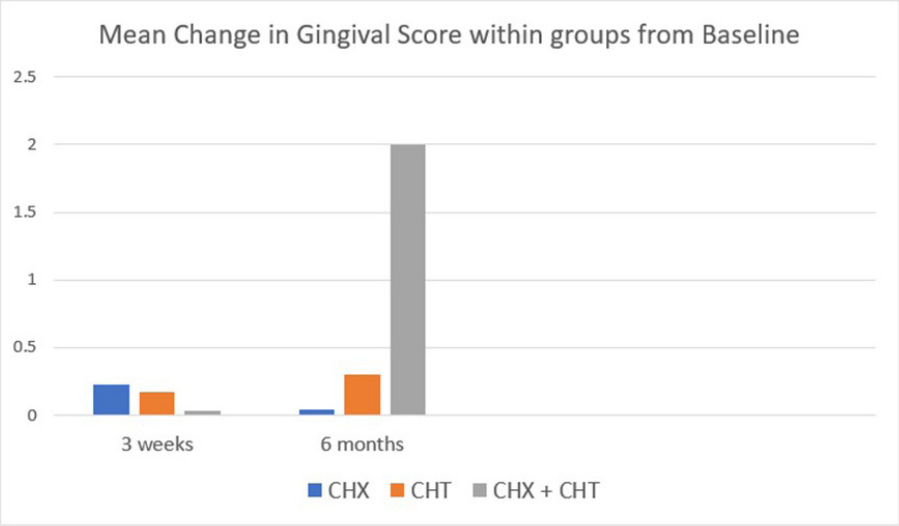

Figure 2. Mean Change in Gingival Score within groups from Baseline

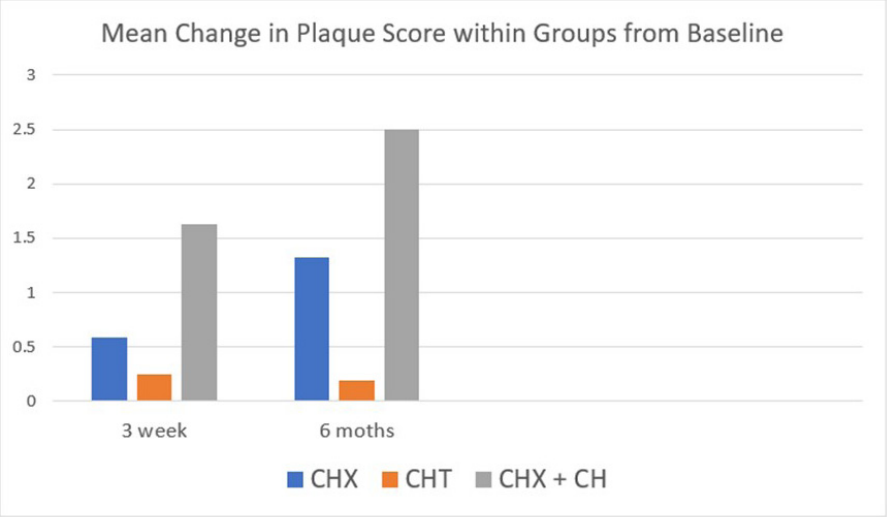

Figure 3. Mean Change in Plaque Score within groups from Baseline

Discussion

It has been reported that combination of chitosan with chlorhexidine in gel form improved the antimicrobial activity of chlorhexidine. A clinical trial by Mhaske et al., (2018), with a short follow period of 4 days, showed that 0.5% CHT + 0.2% CHX mouthwash has increased effectiveness compared to CHX alone. The present study was designed to evaluate the efficacy of combining 0.5% CHT with 0.2% CHX in mouthrinse with longer follow up period.

In the present study, we found statistically significant reduction in plaque and gingivitis level between CHX , CHT and CHX + CHT at all time intervals (p=0.00). On intergroup comparison there were statistically significant differences between all the tested solutions, except between CHX and CHT group which were not statistically significant. However, markedly strong activity was seen in CHX and CHT group (p=0.00) compared to other study groups. This indicates that the use of CHX + CHT combination mouthrinse is highly effective when compared to CHT or CHX mouthrinses alone.

Our results are in agreement with several *in vivo* and *in vitro* studies that have demonstrated the efficacy of CHT and CHX based oral formulations. In a bioadhesion study performed *ex vivo*, it was demonstrated that both the film and gel formulations of chitosan has significant bioadhesive properties and contribute to the retention of the drug in the periodontal pocket as well as release of drug in a prolonged fashion (Ikinci *et al*., 2002). Similarly, results from an *in vivo* study by (Sano *et al*., 2003) are similar to the results of the present study and showed that chitosan rinsing was more effective in reducing plaque formation after a 14 day rinsing period. Further, Uraz *et al*. (2012), also reported that a chitosan mouthwash had a comparable antimicrobial activity to a chlorhexidine digluconate mouthwash, with significant reduction of plaque index . Later, Chen *et al*. (2002), studied the antimicrobial effect of water soluble CHT on *S*. *mutans* and *Candida albicans* counts and reported reductions in bacterial counts of up to 99%. Recently, Costa *et al*. (2014), showed that chitosan mouthwashes possesses a significant antimicrobial and antibiofilm efficiency against oral microorganisms. The toxicological assays performed in this study showed that the chitosan mouthwash possesses no toxicological activity and in reality it was less aggressive to cells than commercial mouthwashes.(Costa *et al*., 2014).

There is one differing *in vitro* report by Vieira *et al*. (2005) on the effectiveness of chitosan based mouthwash formulation on viable microbial count. They demonstrated that CHT did not display any antimicrobial activity due to its neutral pH. Further, the mouthwash formulation used in that study possessed a pH value superior to 6, thus constraining one of the main conditions needed for chitosan to be active – acidic pH (Raafat and Sahl, 2009).

Chlorhexidine digluconate (CHX), has been used for more than three decades for both prevention and therapy of periodontal diseases owing to its bactericidal and bacteriostatic activities (Loe *et al*., 1970). 0.2% CHX has been accepted as the gold standard for its bacteriostatic action for 8-12 hours (Addy *et al*., 2005). The results from our study are in agreement with several studies investigating the efficacy of 0.2% CHX mouth washes similarly reported in literature by the invivo study done by Najafi *et al*. (2012).

With regards to the use of CHX & CHT combination group, our results show that over a period of three months it is more effective clinically when compared to CHT and CHX alone groups. The present study is in agreement with other studies conducted on CHX and CHT formulations. Ikinci *et al*. (2002) evaluated the antimicrobial activity of chitosan with different molecular weight and deacetylation degree as well as their combination with chlorhexidine against the periodontal pathogen, *Porphyromonas gingivalis*. CHX and CHT was shown to have higher antimicrobial activity against *P. gingivalis* (Giunchedi *et al*., 2002) when evaluated CHX buccal tablets prepared using drug loaded CHT microspheres. Combining CHT microspheres as controlled drug delivery systems with CHX not only prolonged the release of CHX in the oral cavity but also improved the antimicrobial activity of CHX. Decker *et al*. **(**2005) evaluated CHX & CHT 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 derivates together with their CHX combination were attached to Streptococci sanguis for 2 minutes. In their results, the CHX & CHT combination was found to be stronger than CHX alone. Similarly, in an *in vitro* study carried out by our research group, on the impact of CHX + CHT formulation on dental plaque bacterial reduction also showed that there was markedly higher and significant activity with CHX & CHT (Vilasan *et al*., 2013). Because chitosan has limited cytotoxicity, no antimicrobial resistance while possessing bio-adhesiveness, anti-inflammatory and antimicrobial properties, it could be considered as a good therapeutic option when used in combination with CHX and would be a valid safer, viable and effective alternative to exsistant mouthwashes.

The exact mechanism for improved results seen with the CHX + CHT combination group is not clearly understood. However, some of the possible mechanisms which could be suggested are: 1) An ionic interaction between the cations due to the amino groups of chitosan and anionic parts of bacterial cell wall such as phospholipids and carboxylic acids has been proposed as the mechanism for the antimicrobial activity of chitosan (Choi *et al*., 1998). 2) Uniting the bioadhesive properties of CHT with the antibacterial activity of CHX would have resulted synergistically in a superior antiplaque

effect to CHT or CHX alone (Harding *et al*., 1999). 3) It has been shown that the amount of chitosan adsorbed on the tissue increases with decreasing cross-linking and adding high molecular weight chitosan has shown maximum minimum inhibitory concentration (Ikinci *et al*., 2002). 4) Chitosan possesses anti-inflammatory effects (inhibits IL-6 and IL-12 production) and also downregulates expression of TNF-alpha and IL-6 at the mRNA level. Furthermore, data reveal that signal pathways activated by lipopolysaccharide (LPS), such as c-Jun NH(2)-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK), can be attenuated by chitosan (Azuma *et al.,* 2015).

The promising outcome of the CHX and CHT combination achieved in our study could be due to: 1) In the trial phase we evaluated the taste of chitosan mouthwash without adding 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 (Costa *et al*., 2014). 2) Storage was done in an amber colour bottle which would have prevented CHT deterioration and increased its shelf life. 3) In this study we attempted to stimulate normal home use conditions as far as possible within the restrictions of the clinical trial to control confounding factors such as the Hawthorne effect, improved oral hygiene, influence of prestudy prophylaxsis, and possible interactions between mouthwash and toothpaste ingredients. However, caution should be exercised when evaluating our results due to several shortcomings. This study was carried out on a small sample size with short evaluation time. Further, we did not carry out any microbiological analysis and toxicity testing. In addition, no attempt was made to find out the exact mechanism of CHX and CHT combination.

Further studies will be of special interest to determine the most effective ratios of CHT/CHX concentrations, to help to optimise new antiplaque formulations. There is need to develop an ideal concentration of CHX in mouthwashes to avoid its unwanted side effects. Future, studies are warranted with larger populations and longer study periods to establish the efficacy of the CHX/CHT combination. Within the parameters of the study, our results demonstrate the clinical superiority of chlorhexidine-chitosan combination mouthrinse as an effective in plaque control compared to chitosan or chlorhexidine mouthwash alone.

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Nil.

Conflicts of interest

There are no conflicts of interest

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