

Effect of Dentifrice Containing Crab-Shell Chitosan on the Accumulation of Dental Plaque in Fixed Orthodontic Appliances Patients: A Randomized Controlled Trial

Abstract

Background: Patients with fixed orthodontic encounter problems in cleaning their teeth and mouths. Dental plaque tends to accumulate on wires, brackets, and surfaces between bonding materials and brackets. Plaque accumulation increases the risks of decalcification, caries, and gingivitis. Chitosan extract is a potential antibacterial agent and inhibitor of dental plaque formation. This study aims to assess the effectiveness of chitosan-containing dentifrice in decreasing plaque accumulation in patients with fixed orthodontic appliances. **Materials and Methods:** This was a prospective, randomized, double-blind study which involved 32 samples (randomly allocated to the control and chitosan groups, after the power of the study was considered) from patients with orthodontic appliances undergoing the last stage of fixed active orthodontic treatment. Plaque accumulation before and after dentifrice use was measured using a plaque index according to O'leary, before and after using toothpaste within 5 days. The patients were instructed to brushing with chitosan-containing dentifrice and chitosan-free dentifrice as a control. Washout period that needed between brushing with chitosan-containing dentifrice and chitosan-free dentifrice as a control was 7 days. Each mouthwash used routinely for 5 days with the same duration and intensity. The data obtained was then analyzed with one-way analysis of variance followed by *post hoc* least significant difference. The level of significance was set as 0.05. **Results:** Plaque accumulation before and after dentifrice use significantly differed ($P < 0.05$) between the control and chitosan groups. The average reductions in plaque accumulation were greater in the chitosan group and control group brushing using chitosan-containing dentifrice. **Conclusion:** Dentifrice-containing chitosan more effectively reduces dental plaque accumulation in patients with fixed orthodontic appliances than dentifrice without chitosan.

Keywords: Chitosan, dental plaque, fixed orthodontic appliance

Introduction

The number of patients seeking orthodontic treatment has increased with the development of esthetic dentistry.^[1] Orthodontic devices are tools that are attached to teeth or several teeth and are surrounded by supporting tissue in an adjustable direction; these devices exert sufficient force on the bone to promote tooth movement during orthodontic treatment.^[2,3] Orthodontic devices can be classified as fixed orthodontic and removable orthodontic appliances. The designs of fixed orthodontic appliances are more complex than those of removable appliances. The complexity of fixed orthodontic appliances complicates oral cleansing and increases the risk of developing plaque accumulation.^[4] Fixed

orthodontic appliances may influence salivary characteristics (i.e., viscosity and secretion) and increase bacterial plaque accumulation at a greater level; these effects consequently promote enamel demineralization, as well.^[5] In addition, the components of orthodontic devices limit the mechanical action of toothbrushes in the removal of plaque from certain areas of the teeth, such as areas between brackets and gingival margins, thus preventing thorough tooth cleaning and promoting plaque accumulation.^[6]

Plaque accumulation during fixed orthodontic treatment can be prevented by maintaining oral hygiene. Brushing teeth with dentifrice is the simplest and most effective way to maintain oral hygiene and is the most generally employed method of oral hygiene in industrialized

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countries.^[7] Antibacterial ingredients are commonly added to dentifrices. Chitosan and modified chitosans are potential antibacterial candidates that inhibit dental plaque formation in teeth.^[8] Chitosan is a natural biomaterial that has been used for biomedical applications. It has various desirable properties, such as biodegradability, biocompatibility, and an antibacterial action against a wide spectrum of bacteria, including Gram-negative and Gram-positive bacteria.^[9] Chitosan, a polysaccharide, is synthesized through the deacetylation of chitin, which is composed of N-acetyl glucosamine units.^[10] Prior studies have confirmed that chewing chitosan-containing gum dramatically controlled populations of cariogenic bacteria.^[11] Moreover, chitosan-containing dentifrice may reduce the enamel decalcification found in patients using orthodontic appliances with poor oral hygiene.^[12] Crab-shell chitosan has the highest chitin content (approximately 65%–70%) among chitosan compounds derived from crustaceans, insects, worms, and fungi.^[13] The fibrous material derived from chitin and its derivatives possesses antibacterial properties.^[14] This study aimed to develop and investigate the effect of dentifrices-containing chitosan derived from crab-shell on plaque accumulation in patients with fixed orthodontic appliances.

Materials and Methods

This study was a double-blind experimental study with a randomized control trial design. This study recruited 32 patients from the Orthodontic Clinic of the Faculty of Dentistry, UGM. The patients were aged 17–25 years, Angle's Class I malocclusion, using fixed orthodontic devices (Straightwire technique), cooperative, and willing

to provide signed written informed consent. A power analysis was performed by G*Power Ver 3.0.10. Software (Franz Faul, Universita't Kiel, Germany). A sample size of 16 patients in each group would present >90% power to detect significant differences with 0.45 effect size and at a significance level of $\alpha = 0.05$ (with regard to 1:1 ratio among two groups). All patients were in the last stage of fixed active orthodontic treatment, using rectangular stainless steel wire 0.016×0.022 (American Orthodontics, USA; to obtain the homogeneous condition in different degrees of crowding severity which can minimize the bias), and had no allergic reaction to any of the ingredients in the tested dentifrices. Patients were excluded if they were receiving preventive treatment or medications that affect the inflammatory status of the gingiva, had systemic disorders or were pregnant or lactating. Dietary instructions were also given, such as, eating three times/day, avoid sticky, gummy, chewy, or very hard food. Patients were divided into two groups, the treatment chitosan and the control groups [Figure 1]. Patients in the chitosan group used toothpaste containing 2% crab shell chitosan extract (Indonesia), whereas those in the control group used toothpaste without 2% crab chitosan extract (Sensodyne, GlaxoSmithKline, Brentford, UK).

The patients were informed of the course of the study and the trained the appropriate method for brushing teeth (following the Horizontal Bass method). Patients were instructed to brush their teeth using a manual orthodontic toothbrush (Oral-B® Orthodontic, Procter and Gamble, Iowa City, USA) for 2 min twice a day by following the horizontal bass method. The patients filled control sheets whenever they brushed their teeth. Dental plaque formation was

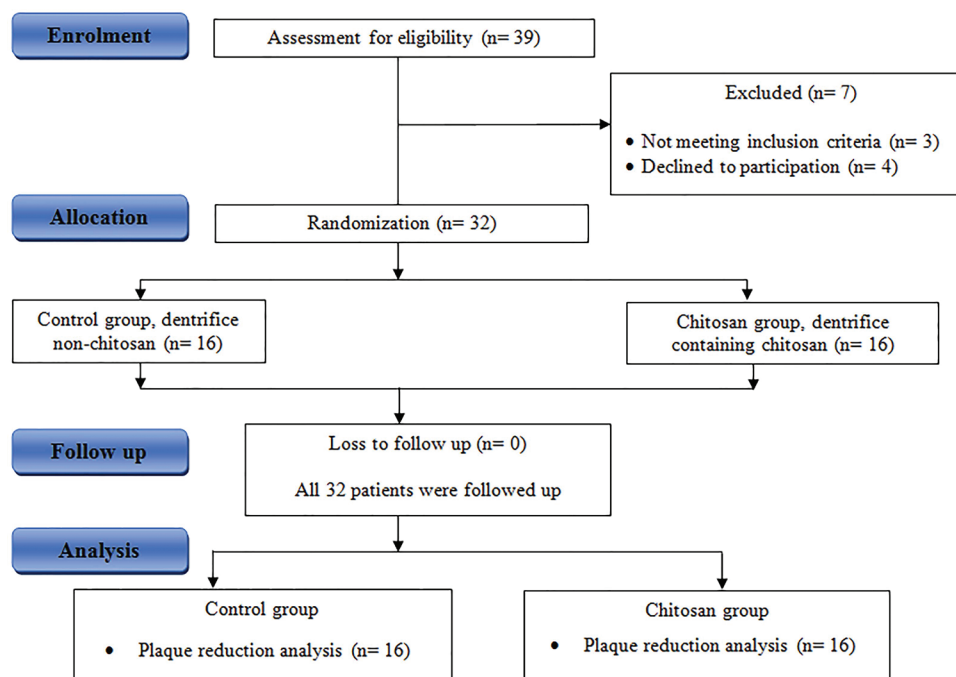


Figure 1: Study flow chart

directly observed in the patient's oral cavity by observing plaque on the tooth surface before treatment on day 0. After initial observation, the patient used dentifrice for 5 days, and plaque formation was observed again on day 6. The time interval between brushing with chitosan-containing dentifrice and brushing with chitosan-free dentifrice was 7 days. After a week of washout period, each group was instructed to use the opposite dentifrice for 5 days, and plaque formation was observed again on day 6. The plaque was assessed in accordance with O'Leary's plaque index.

Patients teeth were rinsed for 30 s with a disclosing solution. The O'Leary index consisted of recording the presence or absence of disclosed plaque on the mesial, distal, buccal, and lingual surfaces of all teeth. After all teeth are examined and scored, the index is calculated by dividing the number of plaque containing surfaces by the total number of available surfaces and then multiply by 100 (percentage). The percentage of the disclosed plaque was then calculated for each patients. All measurements were operated by the two-trained and calibrated observer who were blinded to the applied regimen. The examiners noted an excellent level agreement in their analysis ($\kappa = 0.91$), indicating satisfactory intra-examiner and inter-examiner reliability. Data gathered was analyzed through one-way analysis of variance (ANOVA) followed by *posthoc* least significance difference (LSD). Values of $P < 0.05$ were considered statistically significant. All the data were analyzed using SPSS software Statistical Package for the Social Sciences, (SPSS Inc., Chicago, Illinois, USA).

Results

Plaque accumulation in the oral cavity was initially measured and remeasured after treatment to quantify the effect of treatment on each group. Considering the clinical observation, it has been known that the average reduction in plaque accumulation was greater in the chitosan group than that in the control group [Figure 2]. These findings were supported by the results of plaque assessment. Specifically, the average reductions in plaque accumulation in the chitosan group and the control group were 6.81 ± 4.11 and 4.27 ± 2.02 , respectively [Table 1].

One-way ANOVA was conducted to compare the reduction in plaque accumulation between the control

and chitosan groups. Statistical analysis began with a test for normality through the Shapiro–Wilk test. The results of the normality test indicated that plaque accumulation in the control group and chitosan group decreased by 0.221 and 0.942, respectively. The results of the normality test showed that the significance values of the control and chitosan groups were normally distributed. The Levene test for homogeneity provided the statistical significance value of 0.365 for the control and chitosan groups. These results indicate that the data were homogeneous. The results of the normality and homogeneity test show that the data can be subjected to parametric analysis test through the one-way ANOVA. Table 1 shows the result of one-way ANOVA analysis obtained value of $P < 0.05$. Interestingly, the results of statistical analysis showed that these alterations were statistically significant ($P < 0.05$), and plaque accumulation in the chitosan group was lower than that in the control group [Table 1]. Differences between treatment groups could be determined by *post hoc* test using LSD. The results of the LSD test in Table 1 showed that there were significant differences in group before and after brushing with chitosan-containing dentifrice, group before brushing with chitosan-containing dentifrice and after brushing with chitosan-free dentifrice, group after brushing with chitosan-containing dentifrice and before brushing with chitosan-free dentifrice, and group before brushing with chitosan-free dentifrice and after brushing with chitosan-free dentifrice ($P < 0.05$). Whereas in the group before brushing with chitosan-containing dentifrice and before brushing with chitosan-free dentifrice, group after brushing with chitosan-containing dentifrice and after brushing with chitosan-free dentifrice had no statistically significant difference ($P > 0.05$). These results suggest

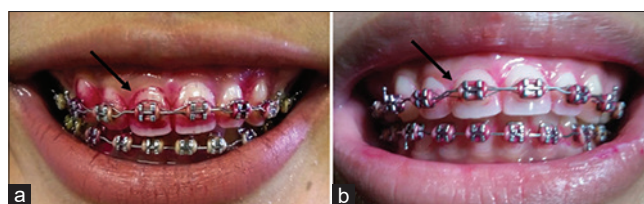


Figure 2: Colored dental plaque after application of disclosing agent solution in patients brushing using chitosan-free dentifrice (a) and brushing with chitosan-containing dentifrice (b). It can be seen that plaque accumulation in Group B lesser than that of Group A

Table 1: Descriptive statistics and results of the analysis of variance and least significance difference tests comparing the plaque index in the four groups tested

Group	n	Plaque index (%)	Significance* (P)	P		
				After Ch	Before C	After C
Before Ch	16	13.14±4.86	0.001	0.001*	0.621	0.001*
After Ch	16	6.33±3.46			0.001*	0.741
Before C	16	12.91±4.89				0.046*
After C	16	8.63±4.91				

Values are presented as mean±SD or P value only by ANOVA, *Significant differences between groups ($P < 0.05$). ANOVA: Analysis of variance; Ch: Brushing using chitosan-containing dentifrice; C: Brushing using chitosan-free dentifrice; SD: Standard deviation

that the use of chitosan-containing dentifrice may reduce dental plaque accumulation of in patients with fixed orthodontic appliances.

Discussion

Maintenance of precise oral health practices is critical for patients who are under orthodontic treatment. Fixed orthodontic appliances promote the accumulation of dental plaque around bracket attachments by complicating the maintenance of dental hygiene. Around 5%–10% of failure in fixed orthodontic treatments are caused by inadequate oral hygiene.^[15] Negligence in keeping proper oral hygiene will result in several negative effects, such as the destruction of periodontal tissue (gingivitis, periodontitis), and further causing deterioration of periodontal health, and affecting the length of orthodontic treatment time as well.^[4] In this study, the use of dentifrices with or without chitosan changed the levels of plaque accumulation. During tooth-brushing, toothbrush bristles exert pressure that removes and reduces food residues and plaque on the tooth surfaces. Abrasive materials and detergent in dentifrices facilitate the removal of plaque, material alba, and residual food debris.^[16] Results of assessment of dental plaque accumulation besides being affected by brushing teeth, may also be affected by the presence of 2% crab-shell-derived chitosan in the dentifrice. The concentration used in this study was 2%. Stamford *et al.* reported that chitosan exerted an optimal inhibitory effect on *Streptococcus mutans* at the concentration of 2%.^[17]

In Indonesia, 342,000 tons of crabs are produced per year. Crab shell is rich in chitosan; approximately reach for 20%–30% of the total weight of crab shells.^[18] Chitosan contains antibacterial substances that can reduce dental plaque formation effectively. Chitosan and is a biopolyamino saccharide with bacteriostatic, bactericidal, and anticalculus properties. It can stimulate salivary flow and secretion, which may consequently promote the self-cleansing of the oral cavity and reduce demineralization.^[19] The antibacterial properties of chitosan are conferred by amine groups, which bond within bacterial cell walls to initiate peptidoglycan hydrolysis and induce the leakage of intracellular electrolytes and constituents; these effects ultimately lead to bacterial lysis or death.^[20] Raafat *et al.* stated that electrostatic interactions influence the initial interaction between polycationic chitosan and negatively charged cell wall polymers. The protective function of bacterial membranes is disrupted when the binding of chitosan to cell wall polymers destabilizes and damages bacterial membranes. The disruption of membrane permeability inhibits the movement of bacterial substances.^[21] Furthermore, a previous *in vivo* study revealed that chitosan inhibits the growth of cariogenic bacteria.^[11] The results of this study are consistent with those of previous studies showing that chitosan was able to reduce the action of plaque formation and periodontitis

by *Aggregatibacter actinomycetemcomitans*, *S. mutans*, and *Porphyromonas gingivalis*.^[22] The results of this work are also in accordance with those reported by Uysal *et al.*, who found that toothpaste containing chitosan can reduce enamel decalcification in patients using orthodontic devices with poor oral hygiene.^[12]

Plaque index score in the group before brushing using chitosan-containing dentifrice with the group before brushing using chitosan-free dentifrice showed no significant differences ($P > 0.05$), whereas plaque index score in the group after brushing using chitosan-containing dentifrice and group before brushing using chitosan-free dentifrice showed statistically significant differences ($P < 0.05$). The result confirmed that the washout process period in this study was successful. Washout period assigned to ensure the effect of previous exposure of first agent or material had been stopped before the second material utilized.

Conclusion

These findings suggest that dentifrices containing crab-shell-derived chitosan may reduce plaque accumulation in patients with fixed orthodontic appliances more effectively than dentifrices without chitosan extract.

Limitation of study

This is a pilot study with a short duration of the study. Longer duration follow-up will give more predictable results and validate the biocompatibility of dentifrices containing crab-shell-derived chitosan in longer period. Furthermore, any confounding factors pertaining to each individual, such as lifestyle, sex, and systemic factors did not controlled in this study. Further studies are required by excluding these confounding factors.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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