Original Article

Evaluation Effect of Chlorhexidine and Green Tea Mouthwash on Saliva pH and Plaque Index

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Abstract

Aims: Applying mouthwash has an important role in reducing caries when compared to other prevention methods. The purpose of this study is to compare saliva pH and plaque index before and after using chlorhexidine mouthwash and a mouthwash with green tea extract. **Materials and Methods:** Forty dental students were randomly assigned to two groups (chlorhexidine and green tea mouthwash). A week before research getting started and during that, the students were asked to use normal diet and Bass brushing method. Salivary pH and plaque index of the participants were measured before, 1 week, and also 2 weeks after the use of mouth washes. Then, the data were analyzed based on Friedman, Mann–Whitney, and repeated-measures ANOVA tests. **Results:** The mean pH was significantly different in the three times of 1 week prior, during, and 2 week after the treatment for the green tea group ($P = 0.01$). However, there was no significant difference in the chlorhexidine group ($P = 0.211$). The mean of new plaque in both chlorhexidine green tea groups was significant ($P < 0.001$). The mean of plaque formation at <48 h in chlorhexidine and in green tea was both significant ($P < 0.001$). **Conclusion:** The effect of chlorhexidine mouthwash and green tea on salivary pH and plaque index was the same in a week. However, the effect of green tea was more than that of chlorhexidine in a 3-week application.

Keywords: Caries, chlorhexidine, dental plaque, green tea, mouth wash

Introduction

Dental caries is a microbial disease that destroys calcareous tooth tissue. The gelatinous mass of bacteria attached to the tooth surface is called dental plaque.[1]

Dental plaque is the most important etiological cause for periodontal diseases.[2] Bacteria in the plaque metabolize the available carbohydrates for energy, and they also produce organic acids as by-products of this reaction. The acid then causes decay by dissolving the crystal structures of the tooth. During the decay process, the pH of the plaque near the tooth surface decreases. The demineralization process of damaged dental structures occurs when the pH of the site increases to 5.5.[1]

Mechanical removal of bacterial plaque biofilm is still recognized as the most accepted plaque control mechanism. The complex bacterial etiology of periodontal disease and dental caries, as well as the inadequacy of using only mechanical plaque control methods, is resulted in considering supportive use of chemical antimicrobial agents as a useful method.[3]

Host modulatory therapy (HMT) has emerged as a new concept for the treatment of periodontal disease. The goals of HMT are to reduce tissue damage and stabilize periodontium by reducing or inhibiting the destructive factors induced by the host response and enhancing protective or regenerative responses.[4] HMT involves the systemic or topical application of drugs, and it is prescribed as part of periodontal treatment.[5] Moreover, it is accepted that using mouthwash and chewing

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sugar-free gum, especially those which contain xylitol alone or in combination with other prophylactics, can increase the acid-neutralizing ability and reduce the process of enamel demineralization.[3]

Among the factors used in HMT treatments are mouthwash. The use of chemical and, recently, herbal mouthwash, along with mechanical methods of cleaning teeth, strengthens the effect of mechanical methods and effectively reduces microbial plaque.[6]

The effect of chlorhexidine over placebos has been proven significant in many studies and has been accepted as a principle.[2,3,6] Other mouthwashes, including the new product namely green tea mouthwash, are among the accepted methods in HMT.

Green tea mouthwash (EGCg) is known as an antimicrobial and prophylactic agent because epigallocatin gamma is effective in reducing acid production in dental plaque.[7]

Ferrazzano *et al*.[8] and Koyama *et al*.[9] proved the anti-caries effects of green tea. Polyphenols in green tea prevent the adhesion of *Streptococcus mutans* damage to the tooth surface by inhibiting the bacterial glucosyltransferase enzyme. Moghbel *et al*.[10] and Balappanavar *et al*.[11] also demonstrated the effect of green tea on reducing plaque.

The aim of this study was to evaluate and compare the effect of chlorhexidine mouthwash and new green tea mouthwash on pH and plaque index. This study is essential due to the fact that dental caries and periodontal diseases are still among the most common oral diseases and most of the treatments are focused on treating these problems, in addition to the introduction of new mouthwash and the insufficient or even contradictory studies at times. The null hypothesis in this study is that chlorhexidine and green tea mouthwash have no effect on the pH and plaque index.

Materials and Methods

In this experimental study, 47 subjects[11] aged between 23 and 26 years with an Decay (D), Missing (M), Filling (F) (DMF) of maximum 10 and minimum 5 were selected. People with active caries, gingivitis and periodontitis or certain systemic diseases, people with orthodontic appliance or under certain medications, people who have undergone periodontal treatment prior to the study, and people who smoke and drink alcohol were excluded from the study. Three people were excluded due to refusing to sign the consent form. Finally, the 44 selected patients were randomly divided into two groups. Furthermore, two cases from each group were lost due to follow-up. Finally, the data from the 40 patients were analyzed. The CONSORT flowchart is presented in Figure 1.

In this study, the aim is to compare green tea with a standard treatment. After institutional approval (number: 23810201931002) and approval by ethics committee (number: 041422349) of the university, informed consent was obtained from the students, they were given a normal diet for a week prior to the study, and they were trained to brush in the Bass method. Then, in all subjects, salivary pH and plaque index were measured using a pH paper and a special plaque index determination kit according to the factory instructions (GC Tri Plaque ID Gel, Japan). A special kit for measuring plaque index based on discoloration on the teeth determines the plaque, which is a change in pink or red color indicates a new plaque, purple or blue color indicates a plaque formed less than 48 h ago, and blue color indicates plaque that is made more than 48 h ago. Salivary pH was also determined based on the color change of a pH indicator paper (Merck KGaA, Darmstadt, Germany).

These subjects were randomly divided into two groups. One group was given 0.2% chlorhexidine mouthwash (Iran Najo Pharmacy, Iran) and the second group was given 0.5% green tea mouthwash (Listerine, Italy). These people were asked to rinse their mouth twice a day, with an equal amount of mouthwash (the size of the bottle cap) for 1 min. During this week, still the Bass method of brushing was applied before using the mouthwash. Also, the subject used normal diet during that period. After 1 week, salivary pH and plaque index were measured again according to the kit instructions. The patients were asked to use mouthwash for another 2 weeks in the same way which was previously instructed, and then 2 weeks later, the patients were asked to be tested for their salivary pH and plaque index. The obtained data were analyzed using Friedman, Mann–Whitney, and analysis of variance (ANOVA) tests by SPSS 20 software (SPSS Inc., Chicago, IL, USA), and the significance level was considered at $P < 0.05$.

Results

The mean pH of saliva before the intervention and 1 week after intervention was not significantly different between the two groups ($P^2 > 0.102$). However, 3 weeks after the intervention, the mean pH in the green tea group was significantly higher than that of the chlorhexidine group ($P^2 < 0.001$). In the chlorhexidine group, the mean pH was not significantly different between three times ($P^1 = 0.211$). But for the green tea group, the mean pH was significantly different between the three times ($P^2 = 0.01$). The result of repeated-measures ANOVA showed no impact of time factor on salivary pH ($P^3 = 0.137$). On the other hand, the changes of pH during the time in both groups were not significant. The effect of time–group interaction was also significant ($P^4 = 0.012$). In other words, the changes of pH between two groups were significant over time [Table 1].

The mean of new plaque index before the intervention and 1 week after the intervention was not significant between the two groups ($P^2 > 0.242$), While 3 weeks after the treatment, the mean of new plaque index in the green tea group was significantly lower than that of the chlorhexidine ($P^2 < 0.001$). In both groups of the study, the mean plaque index was significantly different between the three time measurements ($P^1 < 0.001$).

Also, the mean of plaques formed less than 48 h before intervention and one and 3 weeks after that was significant between the two groups ($P^2 < 0.001$), In the green tea group, the mean plaque index significantly declined between the three time measurements ($P^1 < 0.001$). Moreover, in both groups of the study, the mean plaque index was significantly different between the three time measurements ($P^1 < 0.001$). The mean of plaques formed more than 48 h before the intervention and 1 week after intervention was not significantly different between the two groups ($P^2 > 0.052$). However, 3 weeks after the intervention, the mean of plaques formed more than 48 h in the green tea group was significantly lower than that of the chlorhexidine group ($P^2 < 0.001$). In both groups, the mean plaque index significantly declined between the three time measurements ($P^1 < 0.001$). The result of repeated-measures ANOVA showed that impact of time factor on new plaque and plaques formed less than and more than 48 h were significant ($P^3 < 0.001$). In other words, the changes of plaque index during the time in both groups were significant. The effect of time–group interaction was also significant ($P^4 < 0.001$). In other words, the changes of plaque index between the two groups of study were significant over time [Table 2].

Discussion

The results of the present study indicate that the effect that chlorhexidine mouthwash and green tea had on salivary pH and plaque index was similar and not significantly different in the short term (1 week), and the effect of green tea was significantly higher than chlorhexidine in the long term (3 weeks).

Moghbel *et al*.[10] in their study on three mouthwashes, including 0.2% chlorhexidine, green tea mouthwash with 1%

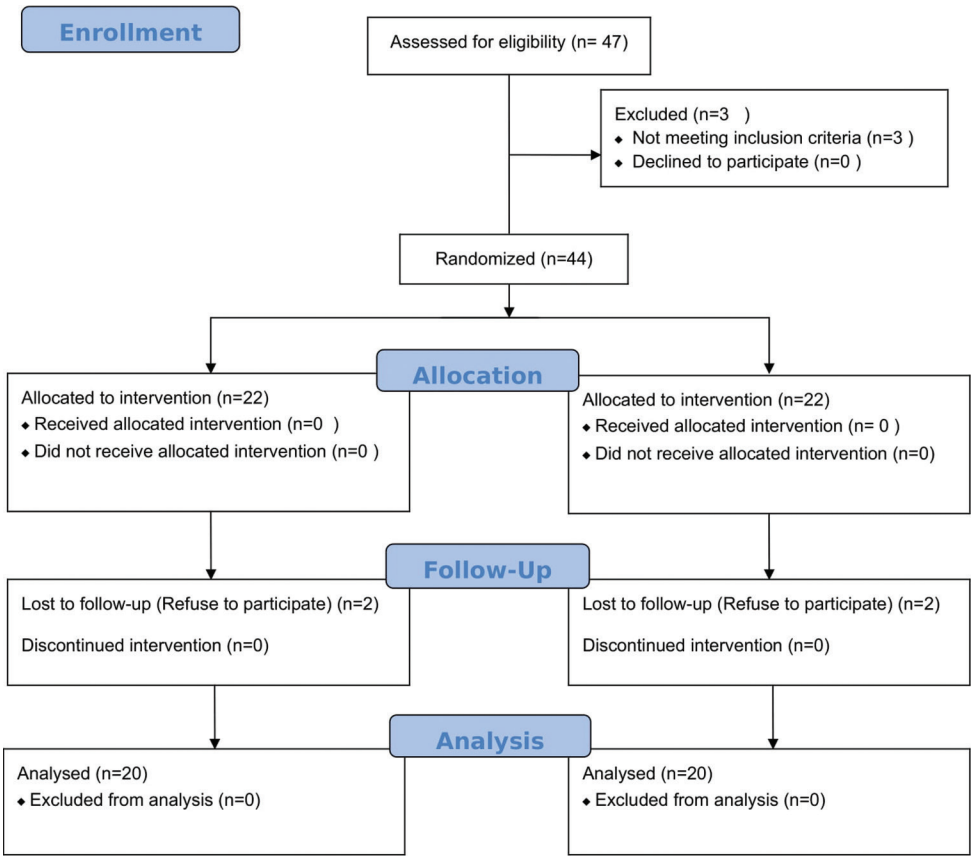

Figure 1: CONSORT table of participation

Table 1: Mean and standard deviation of pH score in the study groups at three time intervals

Time	Mean±SD		P^b	P^c	P^d
	Chlorhexidine	Green tea			
Before intervention	6.88±0.19	6.88±0.17	0.738	0.137	0.012
1 week after intervention	6.84±0.18	6.92±0.11	0.102		
3 weeks after intervention	6.84±0.14	7.03±0.25	<0.001		
P^a	0.211	0.01	-	-	-

[a]*P* within-group analysis: Friedman test, [b]*P* between-group analysis: Mann–Whitney test, [c]*P* effect of time: Repeated-measures ANOVA test, [d]*P* effect of time–group interaction: Repeated-measures ANOVA test. SD: Standard deviation, *P*: *P* value, ANOVA: Analysis of variance

Table 2: Mean and standard deviation of plaque index according to the result of the diagnostic kit in the study groups at three time intervals

Plaque formation	Time	Mean±SD		P^b	P^c	P^d
		Chlorhexidine	Green tea			
New plaque	Before intervention	2.99±0.57	2.77±0.42	0.242	<0.001	<0.001
	1 week after intervention	2.33±0.22	2.36±0.18	0.355		
	3 weeks after intervention	1.69±0.47	0.72±0.25	<0.001		
	P^a	<0.001	<0.001			
Plaques formed <48 h	Before intervention	5.22±0.55	5.9±0.43	<0.001	<0.001	<0.001
	1 week after intervention	2.24±0.17	4.01±0.78	<0.001		
	3 weeks after intervention	3.3±0.46	2.05±0.43	<0.001		
	P^a	<0.001	<0.001			
Plaques formed >48 h	Before intervention	3.03±0.86	2.99±0.79	0.718	<0.001	<0.001
	1 week after intervention	1.91±0.31	1.65±0.53	0.052		
	3 weeks after intervention	1.47±0.32	0.53±0.17	<0.001		
	P^a	<0.001	<0.001			

[a]*P* within-group analysis: Friedman test, [b]*P* between-group analysis: Mann–Whitney test, [c]*P* effect of time: Repeated-measures ANOVA test, [d]*P* effect of time–group interaction: Repeated-measures ANOVA test. SD: Standard deviation, *P*: *P* value, ANOVA: Analysis of variance

tannin, and green tea mouthwash with 10% propylene glycol, showed that there was a significant difference between the use of herbal and chemical mouthwash on bacterial activity. There was also a significant difference between 1% tannin and 10% propylene glycol mouthwash. The finding of their study indicated that green tea with 1% and 10% propylene glycol can reduce the amount of oral anaerobic bacteria by 64%–45%, which is consistent with the results of the present study.

In studies on the anti-plaque effect of chlorhexidine mouthwash compared with oxidizing mouthwash, it was shown that chlorhexidine mouthwash is more effective than oxidizing mouthwash. There was a significant difference between plaque index before and after using chlorhexidine mouthwash for 3 weeks.[12-14] In their study, Fatemi and Sargolzaei[15] compared the effect of chlorhexidine and nanosil mouthwash on gingivitis and reported that the effect of chlorhexidine in reducing plaque index and gingival index was more than nanosil and there was a statistically significant difference between the two mouthwashes.

Rezaei-Soufi *et al*.[16] studying the anti-caries effect of green tea polyphenol extract with 0.05% fluoride mouthwash, 0.2% chlorhexidine, and fluoride–chlorhexidine combination concluded that fluoride–chlorhexidine solution has the most anti-caries effect. Their results indicated that the antiseptic effect of green tea was not significantly different from that of normal saline and also significantly less than chlorhexidine and fluoride mouthwash, which contradicts the results of the present study. Perhaps, the reason for the discrepancy between the results is the different type of method used in Rezaei-Soufi's study. Also in this study, 10% green tea polyphenol extract was prepared by dissolving 10 g of green tea polyphenol extract in 100 mL of dimethyl sulfoxide solution.

In the study conducted by Hirasawa *et al*.[17] on microbial plaque acid production, it was found that salivary pH, measured 30 min after the use of green tea extract, increased significantly and, thus, epigallocatin gallate in green tea reduces the activity of *S. mutans* and the production of acid in dental plaque. Therefore, the results of their study indicated that there is a significant difference between the pH before and after using mouthwash with green tea extract, which is consistent with the present study. Comparing the two mouthwashes, i.e., 0.2% chlorhexidine and a mixed mouthwash containing (0.03% trislucan +0.05% sodium fluoride +5% xylitol), Lakade *et al*.,[18] observed a decrease in the number of *S. mutans* colonies. However, compared to the present study, their study reported that the antimicrobial effect of chlorhexidine was more significant than any other mouthwash.

Comparing chlorhexidine mouthwash and xylitol chewing gum, Nayak *et al*.[19] concluded that chlorhexidine mouthwash was effective in reducing plaque formation; additionally, in their study, Saffari *et al*.[20] concluded that persica could not replace chlorhexidine mouthwash and that chlorhexidine mouthwash had a better effect on plaque index before and after use.

Subramaniam *et al*.,[21] in their study on the effect of green tea on the growth of *S. mutans*, concluded that the use of aqueous or alcoholic solution of green tea leaves inhibits the growth of this cariogenic bacterium and also the inhibitory effect of green tea extract on the growth of *S. mutans* chlorhexidine is 0.2% higher, which is consistent with the present study. In their study, Balappanavar *et al*.[11] compared three mouthwashes, i.e., 0.5% green tea, 0.2% neem, and 0.2% chlorhexidine, and found that all the three mouthwashes were effective in reducing plaque, but 0.5% green tea mouthwash had the greatest effect. Also, the effect of green tea and neem on the gums was more than that of chlorhexidine. In addition, neem and green tea had an effect on increasing the pH of saliva more commonly than chlorhexidine, which is consistent with the results of the present study.

In other studies,[22,23] the effect of chlorhexidine mouthwash on reducing *S. mutans* was proven to be significant. The

superiority of chlorhexidine over other common mouthwashes is due to the mechanism of action of this cationic compound. Chlorhexidine has the potential to destroy membranes and, therefore, can lead to growth inhibition or death of cells. This finding is consistent with the present study.

The results of a study by Mozaffari *et al*.[24] and Yousefimanesh *et al*.,[25] on the antibacterial effect of chlorhexidine and persica mouthwash *in vitro,* showed the ability of chlorhexidine to inhibit the growth of *S. mutans* and *Streptococcus sanguis* and the inability of persica to inhibit the growth of these bacteria. This is also consistent with the findings of the present study.

The limitations of the present study include limited experimental phase and some of the unaccounted personal characteristics of the subject such as salivary concentration, the amount of water that each person drinks daily, and the duration of brushing. It is suggested that more studies be done over a longer period of time and, if possible, people with similar conditions be selected.

Conclusion

Chlorhexidine mouthwash and mouthwash with green tea extract are both beneficial in themselves and effective in increasing pH and decreasing plaque index. Nevertheless, in comparison, the long-term effect that green tea has on salivary pH and plaque index is more significant than that of chlorhexidine mouthwash. It also has fewer side effects than chlorhexidine mouthwash.

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Conflicts of interest

There are no conflicts of interest.

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