ORIGINAL ARTICLE

Effect of Sequential Use of 0.2% Chlorhexidine Mouthwash and Listerine on Microbial Plaque Control

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Abstract

Objective: To evaluate the effects of combined and sequential consumption of chlorhexidine and listerine mouthwashes on plaque indices. **Material and Methods:** Sixteen dental students, both genders, were selected. After prophylaxis, four mouthwash regimens were used, such that in each period, mouthwash was used for 5 days and after each period there was 4 days of washing out. During the mouthwash period, the participants did not use any mechanical plaque control tool. The four regimens included: first regimen, first chlorhexidine then listerine; second regimen, listerine then chlorhexidine; third regimen, only listerine; fourth regimen, chlorhexidine alone. At the end of the period, individuals were evaluated for plaque indices and investigated for bleeding during probing using ANOVA variance analysis and post-hoc Tukey test. The level of significance was set at 5%. **Results:** Regimen 1 with a plaque mean of 0.55 ± 0.25 had significantly lower plaque than other regimens. The maximum rate of plaque was observed in regimen 3. Probing did not cause bleeding in any of the individuals who used the four mouthwash regimens. **Conclusion:** The use of 0.2% chlorhexidine and listerine has the highest effect on plaque reduction.

Keywords: Dental Plaque; Gingivitis; Mouthwashes; Chlorhexidine.

Introduction

Periodontitis is a set of inflammatory diseases affecting the tissues surrounding the teeth and involves the loss of progressive bone around the teeth, and can lead to loose teeth and ultimately loss of them, if left untreated [1]. Periodontitis is caused by microorganisms that stick on the tooth surface and grow and they are associated with the response of immune system against these microorganisms. Actually, inflammation is the most common pathological symptom of periodontal disease and the agent of inflammatory processes is bacterial plaque [2,3].

The etiologic factor of periodontitis is the accumulation of microbial plaque at the dental surface and the gingival line [4]. The prevention of microbial plaque accumulation on teeth surface prevents gingivitis and periodontitis. Mechanical removal of plaque is a primary method in the prevention of periodontal disease, but all patients, even those who do not care about their oral hygiene, cannot completely remove the microbial plaque from the tooth surface using mechanical method. This method requires skill, attention, and patience, as such different methods of chemical control can be used for the microbial plaque in order to complete the mechanical method of its removal. These methods can cause chemical control of the microbial plaque in four ways: (1) containment of the primary microbial accumulation on the tooth surface; (2) inhibition of plaque development and puberty; (3) removal of existing plaques; (4) changes in pathology of plaques [5,6].

The most commonly used chemical methods to locally control plaque are mouthwash, gels, toothpastes, and chewing gum and the use of mouthwashes is one of the most common local methods in chemical control of plaque. Among these mouthwashes, chlorhexidine has been reported to be the most considered recently. Chlorhexidine is one of the most importantly known antiseptics, with good observed results [7].

Chlorhexidine affects a wide range of gram positive and negative bacteria and also some fungi [8]. Previous findings have shown that washing with 10 ml of chlorhexidine gluconate solution 0.2%, twice a day, almost completely stops the progresses of the microbial plaque in humans. Therefore, gingivitis and periodontitis can be prevented and this is confirmed by empirical studies [9,10]. Clinical studies that used chlorhexidine for several months as a mouthwash showed 45 to 61% decrease of the microbial plaque and 27 to 67% decrease of gingivitis. Therefore, it can be concluded that chlorhexidine as a mouthwash is the today most effective substance in controlling plaque [10].

Schiott (1962) was the first person to study plaque inhibition using chlorhexidine, but a decisive study was carried out by Schiott and Leo (1970). They showed that washing with 10 ml of 0.2% chlorhexidine for 1 min twice a day inhibits the growth of the plaque and progress of the gingivitis [11]. Others authors reported that the chlorhexidine molecule reacts with the bacteria on one side and on the other side it reacts with pellicle, thus it prevents bacterial colonization on the tooth surface [12]. In this way, chlorhexidine can have its effect by the strong adhesion to most oral and dental areas. Because this material is released gradually and slowly after adhesion, thus, it provides an antimicrobial environment in a specific time limit. It can also stick to bacterial surfaces and destroy them [13].

The other mouthwash that its clinical impact in reducing the number of microbial plaques has been shown by numerous studies is the listerine mouthwash [14]. Research on the microbiological effects of listerine suggests that it does not cause any changes in the microbial structure of plaque. Listerine is one of the oldest antiseptic mouthwashes in the world market. Various studies have shown that listerine is effective in reducing plaque, preventing caries and forming tartar and preventing gingivitis. This mouthwash does not change the color of teeth and fillings of the composite and its toxicity on fibroblasts in similar concentrations is less than the chlorhexidine mouthwash [15].

In a previous study to compare the anti-gingivitis and anti-plaque properties of chlorhexidine and listerine with an herbal mouthwash, it was concluded that the anti-plaque and anti-gingivitis properties of the herbal mouthwash is better than chlorhexidine and chlorhexidine is better than listerine [16].

Also, some authors in comparing the effects of chlorhexidine, listerine, and meridol on the microbial plaque and gingivitis, also concluded that chlorhexidine mouthwash had a greater anti-plaque and anti-gingivitis properties than listerine and meridol within three weeks without using mechanical methods of oral hygiene [17]. In another research, the effect of sequential use of chlorhexidine and sodium fluoride was studied and the results showed that the sequential use of chlorhexidine and then sodium fluoride reduces the microbial plaque more than using each of them alone or the use of sodium fluoride prior to chlorhexidine [18]. Considering the earlier discussed issues and lack of a similar study on the sequential use of chlorhexidine and listerine mouthwashes, the present study investigates the effects of chlorhexidine and listerine mouthwashes sequences on the clinical index in plaque control.

Material and Methods

Study Design and Sampling

The present experimental study was done in Dentistry Faculty of Tabriz University of Medical Science from September to December 2017. Sixteen dental students (8 females and 8 males) under the inclusion and exclusion conditions, were selected as the participants.

Inclusion and Exclusion Criteria

(1) Subjects signed an informed consent form. (2) Subjects not had gingivitis or periodontitis. (3) Subjects not undergoing radiotherapy in the head and neck region during the previous year. (4) Patients did not receiving antibiotics during the previous 6 months and did not have any systemic condition such as: diabetes affecting the oral health, drug abusers and smokers (smoking ≥10 cigarettes a day), a history of taking biphosphonates, and (5) Subjects did not taking systemic anti-inflammatory agents.

Data Collection

Complete scaling and prophylaxis was done for the participants and the health instructions were given to them. The subjects were clinically examined for a week (twice a day) and the clinical index of plaque index and bleeding during probing in the patients under study before the intervention were measured and recorded by one operator (Kappa intra-examiner = 0.78).

First, cover of two mouthwashes was removed and marked (A and B) then those were given to these individuals. They were asked to do regimen 1 (wash the mouthpiece twice a day (once every 12 h) for 5 days after meals, sequentially with A (chlorhexidine) for 1 min and then with mouthwash B (listerine) for 1 min. During this period, the individuals did not use any mechanical plaque control (toothbrush and dental floss) instruments; after the end of the period, subjects were assessed for index plaque and bleeding during probing. After measuring the aforementioned indices for the individuals, prophylaxis was performed and they were asked to return after four days (the washout period) and they were examined for the absence of microbial plaque and prepared for mouthwash regimen 2.

At this time, first, the mouthwash B, and after 1 min the mouthwash A was used, each for 1 min twice a day. In a similar way, after 5 days, the index plaque and bleeding were recorded while probing and after the washout period, individuals were prepared for mouthwash regimen 3 (only listerine) and again after a washout period they were prepared for the mouthwash regimen 4 (only chlorhexidine) and the results were recorded. It should be noted that four mouthwash regimens were used in different periods, as shown in Table 1.

Table 1. Categorization of regimens used.

Regimen Type	First Mouthwash	Second Mouthwash
Regimen 1	Chlorhexidine 0.2% for a minute	Listerine for one minute
Regimen 2	Listerine for one minute	Chlorhexidine 0.2% for a minute
Regimen 3	Listerine for one minute	-
Regimen 4	Chlorhexidine 0.2% for a minute	-

Statistical Analysis

The obtained data were analyzed using IBM SPSS Statistics for Windows Software, version 17 (IBM Corp., Armonk, NY, USA). The findings were described using descriptive statistics (mean and standard deviation). One-way analysis of variance (ANOVA) test was used to examine the mean difference of index plaque in the regimen groups and Tukey test was used to compare regimens two by two. The significance level in this study was considered at p-value<0.05.

Ethical Aspects

This survey was approved by the Ethical Committee of the Dental School, Tabriz University of Medical Sciences.

Results

Sixteen dental students participated in the study. The average age was 22.18 years, varying from at least 21 to 25 years maximum.

The results show that in regimen 1, the plaque mean was 0.75 ± 0.25, in regimen 2, the plaque mean was 1.50 ± 0.23, in regimen 3 the plaque mean was 3.78 ± 0.22, and in regimen 4, the plaque mean was 1.48 ± 0.41 (Figure 1). ANOVA variance analysis showed a significant difference in the index plaque of the four types of mouthwash regimens (p<0.05).

Figure 1. Comparison of plaque level in four types of mouthwash regimens.

Comparing the regimens two by two to eliminate the index plaque shows that (Table 2). The plaque level of regimen 1 was significantly lower than other regimens (p<0.05); regimen 3 has the highest amount of plaque among regimens (p<0.05); regimen 2 and 4 were similar in plaque level (p=0.99). The rate of bleeding during probing in all the four regimens was zero (there was no bleeding during the whole study period on the students).

Table 2. Comparison of plaque index reduction differences between groups.

Regimens	Mean Difference	p-value	95% Confidence Level	
			Lower Limit	Upper Limit
1 versus 2	–0.74750*	0.000	–0.9781	–0.5169
1 versus 3	–01.0250*	0.000	–1.2556	–0.7944
1 versus 4	–0.73125*	0.000	–0.9618	–0.5007
2 versus 3	–0.27750*	0.012	–0.5081	–0.0469
2 versus 4	0.01625	0.998	–0.2143	0.2468
3 versus 4	0.29375*	0.007	0.0632	0.5243

Discussion

Lately, several studies have been carried out on various mouthwashes and product suppliers have been trying to deliver high quality products with minimal side effects.

The results of the present study showed that regimen 1 (Chlorhexidine 0.2% and Listerine 0.2% for 1 min) with mean of 0.55 ± 0.25 had significantly lower plaque levels than other regimens. The maximum plaque level was observed in regimen 3 (Listerine 0.2% for 1 min). Also, the results of the study showed that there was no bleeding caused by probing in any of the individuals during the use of the four mouthwash regimens.

A study on periodic and reciprocal use of mouthwashes concluded that although listerine can reduce plaque formation, its efficacy is lower than chlorhexidine [19]. Another research reported that chlorhexidine mouthwash, as compared to histerine had more anti-plaque and anti-gingivitis properties within three weeks without the use of oral hygiene methods [17]. The results of earlier studies were also similar to the present study.

The only one study performed on the sequential use of mouthwashes [18] and had a similar method with the present study. These researchers showed that the level of the index plaque and control plaque in individuals differed based on the type and method of mouthwash used and in regimen 1 (sequential use of chlorhexidine and then sodium fluoride) reduces microbial plaque more than when each one is used alone or using sodium fluoride prior to chlorhexidine. In one of the participants, there was no bleeding from the probing when four regimens were used. This is consistent with the results of the present study.

It was showed that the ability of listerine in removing plaque is less than chlorhexidine and that the gingival condition in chlorhexidine is better than listerine [20]. In the present study, in two by two comparison, listerine alone was also observed to have the lowest anti-plaque effect. The plaque levels were less in regimen 3 (Listerine only) together with chlorhexidine, in two types of regimens 1 and 2.

Chlorhexidine has been introduced as a golden standard for comparing the effects of other anti-plaque agents and its efficacy is related to bactericidal and bacteriostatic effects and its durability in the oral cavity. In other words, the positive effect of chlorhexidine is on the high side and side effects are on the lower side, such that it is in the golden standard position [21,22].

A study showed that washing for 1 min twice a day with 10 ml of 0.2% chlorhexidine reduced plaque growth and progression of gingivitis [11]. It was showed that the chlorhexidine molecule reacts on one side with pellicle and on the other side with the bacteria and thus prevents the colonization of bacteria on the tooth surface [12]. So chlorhexidine can be linked and stuck firmly to teeth and most oral areas. As it releases gradually and slowly after attachment, it results in a certain antimicrobial environment within a specific time limit. Similarly, it can stick to bacteria surfaces and destroy them. Also, it should be kept in mind that some of the side effects of this substance are based on this property [13].

Antibacterial property of this mouthwash is because of the destruction of the bacteria cell wall due to its alcoholic base. Another possible cause of antibacterial activity is because it contains positive ion; the cell wall of many bacteria of oral diseases has negative ion and as a result, the mouthwash is absorbed to the cell wall and destroys the cell wall of the microorganisms [21-24]. In addition, chlorhexidine has a low systemic absorption and does not have teratogenic or carcinogenic effects. Chlorhexidine is mainly local and outbreaks when there is change in color of teeth, tingle of mucus, and mucosal desquamative lesions [21-24].

It was showed the effect of reducing the number of aerobic and anaerobic microorganisms by using listerine mouthwash. Plaque index in patients using listerine showed a significant reduction as

compared to previous treatment by mouthwash [25]. Listerine mouthwash results in reduction of plaque, because of its antibacterial properties and the presence of compounds such as Thymol, Eucalyptol, Menthol Salicylate, and Methyl Salicylate [26] and its ability to reduce gingivitis, as reported in chlorhexidine. Therefore, in the present study, there was no gingival bleeding in any of the regimens [27]. The American Dental Association accepts two types of mouthwashes for the treatment of gingivitis: chlorhexidine and essential oil mouthwash containing Thymol, Menthol, Eucalyptol, and Methyl Salicylate [9].

In the present study, the participants used the four mouthwash regimens for 5 days. The lowest plaque mean was observed from chlorhexidine-listerine regimen, which is approximately half of the index plaque level in chlorhexidine regimen alone and it may be stated that these two mouthwashes are in the first regimen, which have synergism effect on each other. May be it is due to the effect of synergy that there was no gingival bleeding in any of the regimens of the present study.

Irsha mouthwash (antiplaque) is an Iranian sample of listerine mouthwash and it is effective in controlling infections or colonies that are predominantly affecting Lactobacillus and Neisseria, Streptococcus salivarius, Streptococcus viridans and Streptococcus mutans, respectively [28]. In a clinical trial study, with a sequential cross-over design, the first group, first used the chlorhexidine mouthwash and then (with a 10-day interval) used Irsha (anti-plaque) mouthwash, and in the second group otherwise was done. None of the subjects used mechanical plaque control while using mouthwash. The results of these investigations showed that the mean of plaque index is significantly decreased after using chlorhexidine mouthwash [29].

Conclusion

The use of 0.2% chlorhexidine for 1 min and listerine for 1 min has the highest effect on reducing plaque. Listerine mouthwash alone has the highest plaque level. The use of listerine regimen for 1 min and 0.2% chlorhexidine for 1 min has the same effect as chlorhexidine alone on plaque.

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