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Antimicrobial effect of Protact mouthwash on Streptococcus mutans isolated from orthodontic elastic rings: A double-blinded randomized clinical trial

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Abstract

Backgrounds: Using mouthwash is an effective complementary approach for maintaining hygiene during orthodontic treatment. This study was conducted with the aim of investigating the antibacterial effect of Protact herbal mouthwash on Streptococcus mutans in fixed orthodontic patients.

Materials and Methods: In this double-blinded clinical trial study, 22 patients with Angle class I malocclusion undergoing orthodontic treatment were selected by census sampling method, and then were divided into two groups: intervention (11) and control (11). The intervention group, used Protact mouthwash and the control group, used 0.2% chlorhexidine mouthwash for 20 days, twice a day and 5 ml each time for 30 seconds. The colony-forming unit of S. mutans adhered to the rings was determined for the samples of both groups using culturing on Mitis Salivarius agar on three stages: prior to intervention, immediately after the intervention and after 20 consecutive days of mouthwash using. $P<0.05$ was considered significant.

Results: In both groups, the average number of S. mutans bacteria isolated from the elastic rings decreased after the intervention compared to before the intervention, and this decrease over time was significant (P<0.001). In addition, there was no significant difference between the two groups of Protact and chlorhexidine mouthwashes at the three stages; prior to the intervention, immediately after the intervention and 20 days after the intervention (P=0.699, P=0.797 and P=0.652, respectively).

Conclusions: Considering the side-effects of long-term use of chlorhexidine mouthwash, Protact herbal mouthwash could be an effective alternative in patients undergoing fixed orthodontic treatments.

Keywords: Mouthwash, Herbal Mouthwash, Chlorhexidine, Wilcoxon test.

Introduction

The use of fixed orthodontic appliances frequently causes an increase in gram-positive flora and cariogenic bacteria, such as Streptococcus mutans, in the oral cavity[1]. Plaque accumulation as a result of fixed orthodontic appliances interfering with oral hygiene maintenance, as well as the irregularity of orthodontic device surfaces, which encourage the growth of the aciduric and acidogenic bacteria, may explain this increase in S. mutans following fixed orthodontic treatments[2]. Patients who use the orthodontic appliances have a larger concentration of this type of bacteria in their saliva and mouths than non-users, which puts them at risk for dental caries[3].

Elastic rubber bands known as orthodontic elastic rings are used to maintain fixed orthodontic wires in their bracket slots. Orthodontic rings increase bacterial adherence, particularly S. mutans, on the teeth and provide new sites for the development of microbial plaque, both of these result in an inflammatory reaction[4]. Due to the regular usage of these rings by orthodontic patients and the inadequacy of mechanical techniques for eliminating bacterial plaque, supplemental protective treatments are required to enhance oral and dental hygiene. Mouthwashes significantly improve oral hygiene and reduce the microbial plaque when used in conjunction with mechanical plaque control methods[5].

Chlorhexidine is one of the most effective mouthwashes in the market and is still regarded as the gold standard for evaluating other anti-plaque agents[6-8] however, long-term use of chlorhexidine is not advised due to a number of side effects, including changes in taste perception, dry mouth and burning sensation, and the production of dental pigments[8]. As a result, more people prefer herbal mouthwashes today[9].

Protact mouthwash, which contains Shirazi thyme, water, glycerin, polyethylene glycol, sodium saccharin, methyl and propyl paraben, is a Lamases family member that is native to Central and Southwest Asia. It is frequently used in alternative medicine in various therapeutic forms such as mouthwash, syrup, tablets, oral capsules, vaginal lotion, and so on[10]. Moreover, thyme extract has been used successfully in the past years to manage oral mucositis[11], relieve aphthous stomatitis[12], and treat common oral fungi (Candida albicans and Candida tropicalis)[13].

On the other hand, Protact has antiseptic, antifungal, antioxidant, spasmolytic, analgesic, and antibacterial effects, the latter of which has received the most research[14]. This herbal product is photochemically and medicinally quite similar to Thymus Vulgaris and can be used in oral and dental disorders in the forms of powder and mouthwash to remove infections and bad breath, as well as to treat toothache and gum discomfort[14]. It also

efficiently decreases S. mutans colonies[14]. The removal of these microorganisms is essential during long-term orthodontic therapy due to the increased risk of tooth cavities and periodontal disease in the presence of increasing microbial plaque.

Considering the side-effects of long-term use of Chlorhexidine mouthwash and the lack of researches on the clinical effectiveness of Protact mouthwash, this study was done to compare the antibacterial effect of Protact with chlorhexidine mouthwashes on S. mutans in fixed orthodontic treatment patients.

Methods

This double-blinded clinical trial study was approved by the ethics committee of Mazandaran University of Medical Sciences with the ethical code IR.MAZUMS.REC.1401.229. The Iranian Registry of Clinical Trials (IRCT) has also approved the investigation with the registration reference code IRCT20170502033770N3. According to the prior studies, the sample size was calculated as 22 individuals[15]. The study population consisted of patients undergoing fixed orthodontic treatment who referred to a private dental clinic in Sari city and met the inclusion criteria but had no exclusion criteria and were selected through a census sampling method during 1400-1401. The inclusion criteria included an age between 13-18 years, patients with Angle class 1 malocclusion and a healthy periodontium without gingival bleeding or any other periodontal diseases[15]. Patients with poor cooperation and a history of allergic reactions to mouthwashes reported by parents, as well as corticosteroids users, patients with severe gingival inflammation during the study and those who had used any antibiotic or mouthwash for 10 consecutive days in the past 3 months were excluded from the study[16-17].

The purpose and the process of the study were explained to the patients, and after obtaining written consent form, they were randomly assigned into two groups (11 patients in each group). The intervention group received Protact mouthwash (Dorsa Group Respinna Pharmaceutical Company) and the control group received 0.2% chlorhexidine mouthwash (Iran Najou Pharmaceutical Company). The patients and the dental assistant were blinded using identical and indistinguishable bottles. Both groups were required to use 5 milliliters of their designated mouthwash for 30 seconds twice a day (at morning and evening) for 20 days, and avoid eating or drinking for one hour after use. All patients were given identical Oral_B toothbrushes and toothpaste, and were taught Horizontal-Scrub method as a proper mechanical hygiene (at least twice a day for 2 to 4 minutes)[15].

Sampling was performed by single clinician in three stages. The first sampling was done 4 weeks after bonding of the brackets (Dentaurum, Germany) on the maxillary left canines and premolars, and the O-rings (American Orthodontics brand) were placed in normal saline solution. For the second sampling, patients were asked to use the designated mouthwash for 30 seconds, and then elastic rings on the maxillary right canines and premolars were immediately removed after rinsing with mouthwash and placed in normal saline solution. Twenty days after the first and second samplings, the third sampling was done following the use of chlorhexidine or Protact mouthwash in the groups. During this sampling stage, elastic rings on the maxillary right canines and premolars were removed and placed in normal saline solution[15].

Then, the samples were transferred to the microbiology laboratory up to 2 hours in an ice-filled container in less

than 4 hours after sampling. The Colony Counting approach was used to determine the effect of mouthwashes on S. mutans. The serial dilutions were first made from the original samples, and these dilutions were subsequently cultivated on Mitis Salivarius agar (Sigma, Germany) containing 20% Sucrose (Sigma) and 200 unit/L Bacitracin (Sigma). The plats were incubated for 48 hours at 37℃ and 10% CO_2. Next, the colony-forming units were compared and assessed among various groups[15].

The data was analyzed by SPSS version 26 using descriptive and inferential statistics. The frequency indices, frequency percentage, mean, and standard deviation were presented in the descriptive statistics section. The Shapiro-Wilk test was employed to examine the normality of variables in the section on inferential statistics. Additionally, analysis of variance, and Mann-Whitney and Kruskal-Wallis tests were performed to assess the hypotheses depending on whether the variables were parametric or non-parametric. The tests' significance threshold was deemed to be less than 0.05.

Results

The Fisher's exact test findings revealed that there was no statistically significant gender difference between the two groups and that the groups were also comparable in this regard (P-value=1). Shapiro-Wilk test failed to confirmed the normality of the data; as such, non-parametric tests were utilized to examine the effect of Protact and chlorhexidine mouthwashes on the amount of S. mutans bacterial colonies isolated from the patients' elastic rings. The Friedman test results are detailed in Table 1.

According to Table 1, there were substantial within-group changes for S. mutans bacteria isolated from patient elastic rings over time and with repeated administration of both mouthwashes (P-value<0.001). The mean number of S. mutans bacteria in both groups was significantly different before intervention, immediately after intervention, and 20 days after intervention. The Wilcoxon test also revealed a difference in the mean number of bacteria in both groups at different time intervals in within-group and pairwise comparisons (P-value=0.003).

The U Mann-Whitney test was performed to assess the effect of the two mouthwashes on the amount of S. mutans bacteria isolated from patient elastic rings (Table 2).

The U Mann-Whitney test results in Table 2 demonstrates a significant difference in the typical number of S. mutans bacteria isolated from elastic rings in patients prior to the intervention (P-value=0.699), immediately after the intervention (P-value=0.797), and 20 days after the intervention (P-value=0.652).

Discussion

Using orthodontic appliances, especially the fixed ones, might increase the level of periodontal bacteria in supragingival and subgingival biofilms and result in inflammation of the periodontium during the treatment[18]. Patients' oral bacterial plaque accumulation rises throughout fixed orthodontic treatment, which may lead to the periodontal disorders, enamel demineralization, and potentially tooth decay. Therefore, it is highly recommended to observe oral and dental hygiene during the treatment period[19]. Mechanical plaque control techniques are particularly beneficial in reducing bacterial biofilms; however, because it is difficult for many of these patients to maintain appropriate oral hygiene using only these techniques, complementing methods must be applied. Using chemical and herbal mouthwashes to control plaque and enhance patients'

oral hygiene during fixed orthodontic treatment is a simple and successful approach[20, 21].

In the current study, the average number of S. mutans bacteria isolated from elastic rings decreased after using both Protact and chlorhexidine mouthwashes, and this decrease was significant over time and also compared to prior the intervention (p-value=0.001). Aghili et al. investigated the antibacterial effect of Shirazi thyme mouthwash on orthodontic elastomeric elastic in an in-vitro study. In this investigation, the antibacterial and antifungal properties of Shirazi thyme extract at concentrations of 1%, 1.0%, 0.01%, 0.001% and 0.0001% were compared with penicillin and nystatin. Their results showed that S. mutans species were significantly eradicated by thyme extract at concentrations of 1% and 0.1% similar to penicillin (P-value=0.01). Aghili et al.'s in-vitro study is congruent with the current clinical investigation as Protact mouthwash contains Shirazi thyme extract[22]. Mirzaei et al. also investigated the antibacterial effect of mouthwashes containing thyme and reported the effect of these mouthwashes against Gram-positive bacteria such as S. Mutans[23].

In this study, the average number of bacterial colonies after 20 consecutive days of mauthwash using significantly decreased from just after the intervention. In Khozeimeh et al.'s study, the antibacterial activity of Shirazi thyme extract against S. mutans was assessed in laboratory conditions at 24, 48, and 72-hour intervals. The results of this investigation revealed that the growth reduction pattern of Shirazi thyme extract is concentration-dependent and time-independent (P-value=0.062), which is in contrast to the findings of the present study. The formulation of Shirazi thyme extract used in the two experiments differed, which may help to explain why the outcomes were different. Furthermore, the duration of time evaluated in Khozeimeh et al.'s study seems to be short, and it is possible that a longer period of more than 72 hours is necessary to examine the time-dependent growth reduction pattern of this extract[24].

The mean number of S. mutans bacteria isolated from elastic rings between patients in the two groups using Protact and chlorhexidine mouthwashes was not significantly different in any of the three sampling stages in this study (P-value<0.05). In a double-blind clinical trial study, Khoshbakht et al. investigated the effects of herbal mouthwashes containing Shirazi thyme, Boswellia thurifera and the combination of these two extracts compared to chlorhexidine in patients with gingivitis. Their findings showed no significant difference between the aforementioned herbal mouthwashes and the control group (chlorhexidine); which is in line with the findings of the current study. Furthermore, chlorhexidine mouthwash showed the most side effects (54.3%), while the herbal mouthwash showed the least side effects (5.7%) and the highest consumer satisfaction (94%)[25]. In a laboratory study, Aghili et al. examined the antibacterial effects of Shirazi thyme and chlorhexidine on microorganisms found on orthodontic elastics. In contrast to the current investigation, Shirazi thyme had a much lower antibacterial impact than chlorhexidine. The type of studies that were carried out, the actual clinical conditions of the study, the oral environment, and numerous other influential factors such as normal oral flora, food consumed during the study, as well as mechanical plaque control methods, which were examined in the present study could be the cause of these disparate findings. In overall, it seems that the

results of the present clinical trial are more reliable than Aghili et al.'s in-vitro study[26].

In this investigation, the average number of S. mutans colonies isolated from elastic rings of patients in the chlorhexidine mouthwash group decreased considerably over time compared to before intervention (P-value<0.001). Additionally, a significant difference in the mean number of bacterial colonies isolated from elastic rings 20 days after intervention and those isolated from elastic rings just after intervention was detected. Chlorhexidine has been used as a gold standard or positive control to evaluate the effectiveness and efficiency of many herbal antimicrobial mouthwashes.

Conclusions

Considering the side effects of long-term use of chlorhexidine and the previous studies' findings, as well as the results of the current study, it seems that the herbal mouthwash "Protact" has a similar effectiveness to chlorhexidine and can be used as a suitable alternative to chlorhexidine in patients, especially those undergoing fixed orthodontic treatments.

The study had some limitations, despite the rigorous application of exclusion criteria to eliminate potential confounders. Diet, which could be a confounder, was neither advised nor controlled. In addition, there are some concerns about maintaining oral hygiene despite comprehensive educating. To evaluate the long-term effectiveness and possible negative effects of the Protact mouthwash in patients receiving orthodontic treatment, additional studies with a longer investigation time are required. Only a limited percentage of patients who met the criteria mentioned under Material and Methods were able to participate in the current investigation, resulting in a restricted sample size. Future studies should include a greater sample size to avoid any bias.

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