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Effects of Persica Mouthwash on Oral Microbiota of Cleft Lip and Palate Patients During Fixed Orthodontic Treatment

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Abstract: The aim of this study was the evaluation of Persica mouthwash on oral microbiota in cleft lip and palate patients before and 6 weeks after fixed orthodontic treatment. In this clinical trial, a convenience sample of seventeen bilateral or unilateral cleft lip and palate patients aged 13-23 years old were selected. Before placing fixed standard edgewise orthodontic appliances, a sample of sublingual saliva was taken from the patients. The patients were asked to use either the Persica mouthwash or not to use any mouthwash for a six week period. Oral microbiota included *Candida albicans*, *Staphylococci*, *E. coli*, Enterobacteriaceae, Gram positive bacilli, *Viridanse streptococci* and *Streptococci mutans* were assessed before and immediately following the experiment. This study showed that after 6 weeks in 44.4% of cleft lip and palate patients who used Persica mouthwash the level of *Candida albicans* increased compared with 0.0% of patients who were not Persica users (p-value = 0.03). Comparison of other microorganism changes after 6 weeks in 2 groups was not statistically significant (p>0.05). Persica mouthwash can not alter oral microbiota in cleft lip and palate patients during a six-week period except for *Candida albicans* which can increase the growth of it.

Key words: Oral microbiota, fixed orthodontic treatment, cleft lip and palate, persica mouthwash

INTRODUCTION

Clefting is one of the most common major congenital defects in humans. As a result of modified anatomy, cleft patients are more susceptible to dental diseases that might be increased after initiation of orthodontic treatment (Lucas *et al.*, 2000; Ahluwalia *et al.*, 2004).

Knowledge about the degree of contamination in cleft patients before and during orthodontic treatment may lead to appropriate preventive therapy.

Several reports indicate that some plants traditionally used as herbal toothbrushes, contain antimicrobial agents that could enhance their effectiveness as oral hygiene aids (Al-Lafi and Ababneh, 1995; Al-Baghieh *et al.*, 1994; Almas, 1999; Almas and Al-Lafi, 1995).

In Moslem countries, chewing sticks are often referred to as miswak and their use is considered as a religious practice (Almas, 2001).

Salvadora persica, a very popular plant in the middle east, contains a number of identified antimicrobial and other prophylactic components including fluoride

(Al-Lafi and Ababneh, 1995), alkaloids (Khalessi *et al.*, 2004), sulphur compounds (Ezmirly *et al.*, 1979), glucosinolates (Darmani *et al.*, 2006) and volatile oils such as benzyl isothiocyanate (Khalessi *et al.*, 2004).

It has been demonstrated that extracts of miswak resulted in improved gingival health and inhibited growth of cariogenic bacteria (Khalessi *et al.*, 2004).

Another study showed that *Streptococcus mutans* was more susceptible to miswak antimicrobial activity than *Lactobacilli* (Almas and Al-Zeid, 2004).

In this regards, Almas *et al.* (2005) compared antimicrobial activity of eight commercially available mouthrinses and 50% miswak extract against seven microorganisms. They found that mouthrinses containing chlorhexidine had the maximum antibacterial activity, while miswak extract had low antibacterial activity.

Additionally, Sforata *et al.* (2007) showed that mouth rinsing with miswak extract, compared with water rinsing, resulted in protracted elevation of plaque pH and the difference between two groups was statistically significant at 30 min.

Since, patients with cleft are at a significant risk for caries and periodontal diseases, current study was designed to determine whether topical application of Persica mouthwash influences oral microbiota in the cleft lip and palate patients.

MATERIALS AND METHODS

A convenience sample of 17 bilateral or unilateral cleft lip and palate patients aged 13-23 years old (11 males and 6 females) referring to cleft lip and palate clinic of Mashhad Dental School were selected.

The study was approved by University of Mashhad Ethics Committee.

Before starting the study, all the patients were examined for dental caries and decayed teeth were filled if there were any. Then the Modified Bass technique was taught to all the patients for tooth brushing.

Exclusion criteria included the presence of prosthodontic appliances or significant systemic diseases causing xerostomia. Also, all the patients who were on antibiotic therapy 4 to 6 weeks before starting the trial were excluded.

Before placing fixed orthodontic appliances, a sample of sublingual saliva was taken from the patients seated on the dental unit in the upright position. The reason for selecting sublingual saliva was not only due to better accumulation of saliva but also because of existing whole saliva in this area. The patients were asked not to eat foods especially those with high sugar or high acidity. They were also asked not to smoke or to chew gum 2 h before saliva sampling. The time of saliva collection was at least 2 h after breakfast.

Then patients were randomly divided into two groups; Group 1 who used Persica mouthwash twice daily for 6 weeks (9 patients) and Group 2 who did not use any mouthwash (8 patients).

Persica mouthwash was provided by Pursina Ltd (Tehran, Iran) and the patients were instructed to dilute 15 drops of mouthwash in 15 mL of water and keep in the mouth for 20 sec before expectoration. They were told to do this twice daily for 6 weeks according to manufacturer's instructions.

Throughout the trial, the patients followed their usual daily oral hygiene measures (brushing and flossing) in addition to application of the assigned mouthwash in Group 1.

Six weeks after placing fixed orthodontic appliances, the second sample of saliva was taken from both groups.

The microbial samples were obtained after the subjects had chewed a piece of paraffin wax for 1 min to stimulate salivary flow, then small amount of pooled saliva in the sublingual area was taken using a sterilized swab.

In this technique end of swabs were placed in the sublingual mucosa and after saliva collection, was transferred to a tube containing trypticase soy broth and incubated at 37°C for 2 days. Then the samples were cultured in blood agar and MacConkey agar for 24 h and Gram stained. MacConkey agar is an agar on which only Gram-negative bacteria can grow. What is more is that *E. coli* will grow into red colonies, as there is a pH indicator present. MacConkey's is a selective medium that inhibits the growth of Gram-positive bacteria due to the presence of crystal violet and bile salts. Gram-negative bacteria grow well on MAC. Whenever bacterial colonies grow on MacConkey's agar, they are certainly Gram-negative ones (since Gram positive bacteria do not grow on this type of medium). If the colonies are pink, they are Gram- lactose-fermenting bacteria. These pink colonies are typically of the Enterobacteriaceae family.

However, blood agar contains blood cells from an animal (e.g., a sheep) and most bacteria will grow on it.

Then samples were cultured in CTA containing monitol and sorbitol for definite diagnosis of *Streptococcus mutans*. In this study we assessed *Streptococcus mutans*, *Candida albicans*, *Staphylococci*, Gram positive bacilli and other microorganisms. Then saliva was cultured in CTA included monitol and sorbitol.

When gram-positive cocci were observed on microscopic evaluation, catalase-test was performed by using 3% H₂O₂ to determine the presence of *Staphylococci* or *Streptococci*.

Staphylococci are catalase-positive and *Streptococci* are catalase-negative.

The catalase-negative cocci were gram stained and evaluated by microscope. If they were diagnosed as *Streptococci*, following tests would be done:

- Hemolysis test: Based on this test the *Streptococci* have three hemolysis forms, α , β and γ and *Streptococcus mutans* has α or β hemolysis forms

In present study, we removed α and γ colonies and put them in CTA culture including 1% monitol and sorbitol that changed the color of two cultures to yellow. After that the data was analyzed by Chi-square and Mann-Whitney tests.

RESULTS

According to Table 1 and with Chi-square test, type of cleft is similar in both groups (p-value = 0.09).

Table 2 shows the study population according to sex (p-value = 0.85).

Figure 1 shows that after 6 weeks the level of *Streptococcus mutans* in 11.1% of Group 1 patients who

Table 1: Frequency distribution of study population according to cleft type

Cleft type	Group 2		Group 1		Total	
	No.	%	No.	%	No.	%
Unilateral	3	37.5	7	77.7	10	58.8
Bilateral	5	62.5	2	22.2	7	41.2
Total	8	100.0	9	100.0	17	100.0
Chi-square test (χ^2)	2.83					
p-value	0.09					

Table 2: Frequency distribution of study population according to sex

Sex	Group 2		Group 1		Total	
	No.	%	No.	%	No.	%
Male	5	62.5	6	66.7	11	64.7
Female	3	37.5	3	33.3	6	35.3
Total	8	100.0	9	100.0	17	100.0
Chi-square test (χ^2)	0.32					
p-value	0.85					

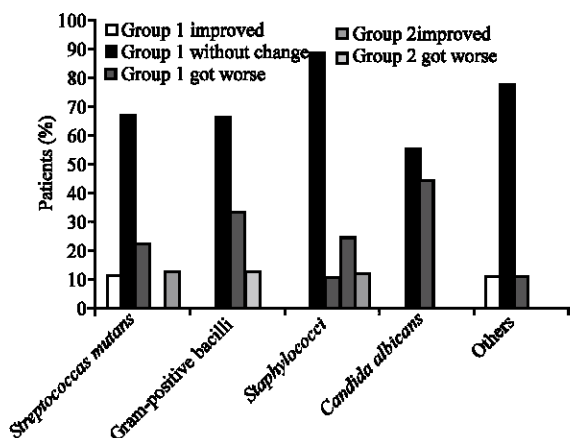


Fig. 1: Percentage of changes in microorganisms levels in patient's saliva after six weeks following the introduction of orthodontic appliance

used mouthwash decreased, in 66.7% of them remained unchanged and in 22.2% increased but at the same time level of *Streptococcus mutans* in 0.0% of patients in Group 2 decreased, in 87.5% remained unchanged and in 12.5% increased ($p = 1$).

According to Fig. 1, after 6 weeks the level of Gram-positive rods in 0.0% of Group 1 patients decreased, in 66.7% of them remained unchanged and in 33.3% increased but at the same time level of bacilli in 12.5% of patients in Group 2, decreased, in 87.5% remained unchanged and in 0.0% increased ($p = 0.05$).

As a comparison after 6 weeks the level of *Staphylococci* in 0.0% of Group 1 patients who used mouthwash decreased, in 88.9% of them remained unchanged and in 11.1% increased but the level of *Staphylococci* in 25% of patients in Group 2, decreased, in 62.5% remained unchanged and in 12.5% increased ($p = 0.33$).

Figure 1 shows that after 6 weeks in 44.4% of Group 1 patients the level of *Candida albicans* increased

compared with 0.0% of Group 2 patients who did not use Persica mouthwash (p -value = 0.03).

Comparison of other microorganism changes after 6 weeks in two groups was not statistically significant.

DISCUSSION

The main aim of this study was the evaluation of Persica mouthwash effects on oral microbiota in the cleft lip and palate patients. The results indicated that the Persica mouthwash didn't alter the level of many microorganisms significantly.

We know that *Streptococcus mutans* are generally considered the major etiologic agents for dental caries. The *Streptococcus mutans* preferentially colonize human teeth surfaces and prosthetic appliances and especially colonize retention sites (Jordan and LeBlanc, 2002). The present study did not reveal significant changes in level of *Streptococcus mutans* after using Persica mouthwash which is possibly due to modified oral cavity anatomy of cleft lip and palate patients that creates more retention sites for accumulation of microorganisms.

Present finding differ from Khalessi's investigation reporting significant reduction in the carriage rate of *Streptococcus mutans* after using Persica mouthwash (Khalessi *et al.*, 2004).

On the other hand, *Staphylococci* are not usually considered to be members of the resident oral microbiota but they may be present transiently. Interestingly this is in contrast to other surfaces of the human body in close proximity to the mouth such as the mucous membranes of the nose where they are among the predominant components of the microbiota.

In cleft lip and palate patients, because of modified oral cavity anatomy the transition between oral, nasal and skin flora occurs. In this study there was no significant difference after 6 weeks in level of *Staphylococci* between 2 groups but at the same time the level of *Candida albicans* was significantly increased in patients who used Persica mouthwash and this is in contrast to Al-Baghieh *et al.* (1994) study who reported that Persica inhibits growth and acid production of *Candida albicans*. They showed that the extract had a fungistatic effect for up to 48 h at a concentration of 15% and above. Although, there is limited information about active compounds contributing to antibacterial properties of miswak, this antimycotic effect could be related to one or more of the root contents of Persica such as chlorine, trimethylamine, and alkaloid resin and sulphur compounds.

The increase of *Candida albicans* in the present study is difficult to explain but could be due to the fact that because of modified oral anatomy in cleft lip and

palate patients we need more concentration of Persica mouthwash to show significant antimycotic effects.

Gram positive rods such as *Lactobacilli* are commonly isolated from the oral cavity although they usually comprise less than one percent of the total cultivable microbiota.

They are highly acidogenic organisms and are associated more with carious dentin and the advancing front of carious lesions than with the initiation of the disease. In this study there was no significant difference between two groups after 6 weeks (p-value = 0.05).

These findings provide support to justify a larger, long term clinical trial to assess further potential oral health benefits of Persica mouthwash.

CONCLUSION

Persica mouthwash can not alter oral microbiota in cleft lip and palate patients during a six-week period except for the *Candida albicans* that can increase the growth of it. Therefore, special strategies should be designed for these patients to prevent caries.

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