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Comparison of the Antimicrobial Effects of Persica Mouthwash and 0.2% Chlorhexidine on Aggregatibacter Actinomycetemcomitans of Healthy Individuals and Patients with Chronic Periodontitis

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Abstract: The usage of antimicrobial oral rinses plays an important role in maintaining oral hygiene mainly by reducing the numbers of dental plaque microbes. Mouthwashes are very useful in the reduction of microbial plaques. The goal of this study was to compare the antimicrobial effect of 0.2% Chlorhexidine (CHX) and Persica Mouthwashes (PM) on *Aggregatibacter actinomycetemcomitans* of healthy individuals and patients with chronic periodontitis. The subject of the study was formed for 32 volunteers. They were divided into two groups according to their periodontal status. Each group is composed of 16 periodontally healthy individuals and 16 patients with chronic periodontitis. Each of this group was further subdivided randomly into two groups based on their use of 0.2% CHX and PM. Subgingival microbial samples were collected from two deepest pockets by inserting a sterile paper point in each pocket for 60 sec before and 30 sec after then, 30, 60, 120 and 300 min after using mouthwashes. The numbers of *A. actinomycetemcomitans* colonies available in subgingival crevicular fluid were determined in specific culture medium (Trypticase soy agar bacitracin-vancomycin). The numbers of bacterial colonies then were compared in two groups. The result showed that the rate of reduction at different time intervals was not statistically significant ($p = 0.05$) in both groups of patients. PM and 0.2% CHX had antimicrobial effects against A.

Key words: 0.2% Chlorhexidine, persica, *Aggregatibacter actinomycetemcomitans*, dental plaque, oral hygiene, Iran

INTRODUCTION

The usage of chemical plaque control back at least 6000 years ago (Fischman, 2000). In the middle east, *Salvadora persica* is a plant, contains many components like florida, alkaloids, sulphur, glucosinelates and benzyl sothiocyanate (Al-Lafi and Ababneh, 1995; Ezmirly *et al*., 1979; Darmani *et al*., 2006; Khalessi *et al*., 2004).

The main ingadienent of persica is miswak improved gingival infolammation and prevented growth of some cariogenic bacteria (Khalessi *et al*., 2004).

Among available mouthwashes, chlorhexedine was shown to be highly effective in the reduction of dental plaque and pathogenic organisms including *Streptococcus mutans* (Salehi and Sh, 2006).

The bacterial etiology of chronic periodontitis is complex with a variety of organisms responsible for the inhibition and progression of disease. Although, over 400 different bacterial species have been implicated as periodontal pathogens, many of these organisms may also be present in periodontally healthy individuals and can exist in communal harmony with the host (Salari and Kadkhoda, 2004).

Good oral hygiene practice has always been an important aspect of total health care. Maintaining oral health offers many advantages which most people seldom appreciate and fully maximize (Rao *et al*., 2001). Relatively few studies have been assessed the effect of percica mouth wash on the oral microbiota (Salehi and Sh, 2006; Mortazavi and Balali, 2001; Jajarm *et al*., 2009).

The main thrust of this study was to compare the antimicrobial effects of 0.2% Chlorhexidine (CHX) and Persica Mouthwashes (PM) on the oral microbiota of periodontally healthy individuals and patients with chronic periodontitis.

Knowledge gained from this study could be beneficial in helping patients with chronic periodontitis to be treated appropriately, particularly after periodontal surgery and to ensure a faster healing process. Furthermore, this study would be of great help to healthy

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individuals to increase their oral health awareness in the control and prevention of infections caused by *Aggregatibacter actinomycetemcomitans*.

MATERIALS AND METHODS

This experimental study involved thirty two patients. After asking them to fill up the inform concept, they were divided according to the periodontal status into sixteen periodontally healthy individuals and sixteen patients with chronic periodontitis. The periodontal status was established according to clinical parameters proposed by the World Health Organization (WHO). The clinical finding for periodontally healthy individuals include: pink, not red or swollen; probing depth <3.0 mm; absence of gingival bleeding and supra or subgingival calculus.

Common clinical findings for patients with chronic periodontitis included erythema, edema, tissue enlargement, point bleeding and it also involves clinically detectable levels of host tissue destruction. These include bone loss and attachment loss ≥4 mm. Exclusion criteria were smokers, patients with history of systemic disease, periodontal treatment within the past 6 months, female patients on their pregnancy and lactation, individuals with orthodontic and/or prosthodontic devices, patients who had taken antibiotics within the last 3 months for 10 consecutive days and patients with adverse reaction to CHX and PM.

Patients were randomly divided into two groups in their use of CHX and PM. Following clinical examination, subgingival microbial samples were collected from the deepest pocket and at the 30 sec, 30, 60, 120 and 300 min after completing each one of the following protocols applied under supervision:

- About 30 sec mouthrinse with 15 drops of PM in 5 mL of water for eight subjects with healthy periodontium and eight patients with chronic periodontitis
- About 30 sec mouthrinse with 15 mL 0.2% chlorhexidine 8 subjects with healthy periodontium and 8 patients with chronic periodontitis
- The patients/respondents were not allowed to eat or drink anything for 1 h prior to the collection and during the course of the experiment

Instrument for gathering data and validation: This instruments/equipment used for data gathering and validation are the following: paper point, microtest tube, petri dish, incubator, test tube, stereoscopic microscope and light microscope.

Procedure for gathering data

Subgingival microbial sample collection: First sample collection area was isolated with cotton rolls, cleaned with sterile cotton pellets, then air dried. Two sterile paper points were inserted into the bottom of two deepest pockets for 60 sec. Samples were collected before at the 30 sec, 30, 60, 120 and at 300 min after completing each one of the following protocols applied under supervision:

- About 30 sec mouthrinse with 15 drops of PM in 15 mL of water for 8 subjects with healthy periodontium and 8 patients with chronic periodontitis
- About 30 sec mouthrinse with 15 mL of 0.2% CHX 8 subjects with healthy periodontium and 8 patients with chronic periodontitis

Then, the paper points were placed into microtest tubes containing 1.5 mL reduced Ringer's solution and kept at 4°C until the processing procedure was carried out. Next the processing was performed within 2 h. The samples were mixed well by vortex for 60 sec. Then, 0.1 mL of each of the samples were placed in agar plates in duplicates using Trypticase Soy Bacitracin Vancomycin (TSBV) agar plates, containing 1 g L^{-1} yeast extract, 100 mL L^{-1} horse serum, 75 mg L^{-1} bacitracin and 5 mg L^{-1} vancomycin. Afterward the plates were incubated at 37°C for 5 days in an environment containing 5% CO_2. Colonies of *Aggregatibacter actinomycetemcomitans* were identified by morphology in stereoscopic microscopy. They were identified as small, rough, round, convex, translucent and adherent colonies with stellate central structure.

Finally in order to confirm the results, Gram staining and biochemical tests of glucose, fructose and mannose fermentation as well as catalase reaction were performed for the positive colonies of A.a. The method for counting of the bacteria was pour-plate method.

Statistics for analyzing data: The statistical analyses used in this study were t-test and ANOVA.

RESULTS AND DISCUSSION

The *A. actinomycetemcomitans* colonies were identified and pour plate method was used with appropriate dilutions to count the Colony Forming Units (CFUs). A dilution of 1:10 of the sample was cultivated in Trypticase Soy Agar Bacitracin Vancomycin (TSBV) media. The tests were conducted 3 times for each sample and the mean CFU count was determined. A p-value <0.05 was considered statistically significant. The t-test was

used to compare the two mouthwashes in this study. To compare the reduction in the number of the *A. actinomycetemcomitans* at 30 sec, 30, 60, 120 and 300 m after using mouthwashes ANOVA was used. The results show that there was a significant reduction in the number of *A. actinomycetemcomitans* after the use of PM at 30 sec ($p = 0.02$), 30 min ($p = 0.02$), 60 min ($p = 0.02$), 120 min ($p = 0.02$), 300 min ($p = 0.05$) after use of mouthwash. Moreover, the maximum reduction appeared to occur 30-120 min after use of the mouthwash. However, the rate at which PM was able to reduce the number of *A. actinomycetemcomitans* among the times tested was not statistically significant. (ANOVA; $p = 0.99$). These results also suggest that the greatest antimicrobial capacity of PM lasted for only a relatively short time (Until about 120 min). Beyond 120 min the effect of PM appeared to diminish slightly. After the use of 0.2% CHX, there was a significant reduction of *A. actinomycetemcomitans* at 30 sec ($p = 0.00$), 30 min ($p = 0.05$), 60 mim ($p = 0.05$), 120 min ($p = 0.05$), 300 min ($p = 0.05$) after use of the mouthwash. This indicates that 0.2% CHX was able to significantly reduce the number of *A. actinomy-cetemcomitans* but to a much lesser extent compared to PM. In addition, the maximum reduction appeared to be at 120 min after use of the mouthwash. Similarly, the rate at which 0.2% CHX was able to reduce the number of *A. actinomycetemcomitans* was not statistically significant among the time intervals (ANOVA, $p = 1$). These results also suggest that 0.2% CHX may have longer substantivity effect on the *A. actinomycetemcomitans* constantly diminish slightly at 300 min. Overall there was significantly more reduction after use of PM at 30 sec, 30, 60 and 120 min (t-test; $p = 0.04$) compared to 0.2% CHX.

A comparison between the efficacy of PM in percentage reduction of *A. actinomycetemcomitans* colonies in periodontally healthy individuals and chronic periodontitis patients. The results indicate that PM had almost the same rate of reduction in both groups. This may be explained by the fact that there are significantly more numbers of *A. actinomycetemcomitans* isolated from patients with chronic periodontitis before using the mouthwash compared to healthy subjects and therefore, their numbers are more readily decreased in healthy subjects. The finding have been shown in Table 1and 2. Relatively few studies have been compared the effect of persica and chlorhexidine mouth washes on the oral microbial colonies. Salehi and Sh (2006) compared the antimicrobial effects of chlorhexidine with persica mouth wash in fixed orthodontics patients (Salehi and Sh, 2006). Mortazavi and Balali (2001) assessed the effect of persica and many other mouthwashes on the reduction of

Table 1: Reduction of *A. actinomycetemcomitans* (%) after using Persica Mouthwash (PM) in periodontally healthy subjects and patients with chronic periodontitis

	Reduction in the number of *A. actinomycetemcomitans* (%)	
Time interval	Healthy subject	Patients with chronic periodontitis
30 sec	96.60	87.66
30 min	98.00	99.50
60 min	98.00	99.50
120 min	98.86	99.50
300 min	78.85	97.71

Table 2: Reduction of *A. actinomycetemcomitans* (%) after using 0.2% CHX mouthwash in periodontally healthy subjects and patients with chronic periodontitis

	Reduction in the number of *A. actinomycetemcomitans* (%)	
Time interval	Healthy subjects	Patients with chronic periodontitis
30 sec	63.11	74.14
30 min	71.74	50.00
60 min	66.89	43.10
120 min	65.05	60.34
300 min	60.39	38.79

mutants streptococci (Mortazavi and Balali, 2001). Jajarm *et al*. (2009) assessed the effect of persica on oral microbiota in cleft lip and palate patients with fixed orthodontic treatment (Jajarm *et al*., 2009).

The difference in the finding of the present study with the reported studies (Salehi and Sh, 2006; Mortazavi and Balali, 2001; Jajarm *et al*., 2009) is presumably due to the difference in the method of the study and time of mechanical and chemical control protocol and variation categories of microorganism and variation in laboratories protocol as well as individual people with different disease.

The results show that PM significantly reduced the number of *A. actinomycetemcomitans* at 30 sec, 30, 60, 120 and 300 min ($p = 0.001$) after use of the mouthwash. Moreover, the maximum reduction appeared 30-120 min after rinsing with the mouthwash. However, the rate at which PM rinsing was able to reduce the number of *A. actinomycetemcomitans* between the times tested was not statistically significant (ANOVA; $p = 0.98$). The results also suggest that PM was more potent in reducing the of *A. actinomycetemcomitans* colonies up to 120 min although, the maximum substantivity is also limited to a short time (120 min). Beyond 120 min the effect of PM appeared to diminish slightly. Similarly, for 0.2% CHX there was a significant reduction in numbers of *A. actinomycetemcomitans* at 30 sec ($p = 0.01$), 30 min, 60 min ($p = 0.01$), 120 min ($p = 0.01$), 300 min ($p = 0.05$) after use of 0.2% CHX. The greatest reductions happened at 30 sec and 120 min after using the mouthwash

indicating that the maximum antibacterial effect of 0.2% CHX occurred during those times. As with the previous results, the rate of the reduction of 0.2% CHX at different time intervals was not statistically significant (ANOVA; p = 0.39). These results also suggest that 0.2% CHX had shorter substantivity effect as the antimicrobial capacity was decreased at to 300 min with a immediate increase at 30 sec after using the mouthwash. When the antimicrobial effect of PM and 0.2% CHX were compared i.e., their ability to reduce the numbers of *A. actinomycetemcomitans*, there was greater reduction after use of PM at 30 sec and 30, 60, 120 and 300 min compared to 0.2% CHX. On the basis of the finding the Ho is rejected.

Likewise, Fernandes-Naglik *et al.* (2001) and Jenkins *et al.* (1989) reported that the antibacterial activity persists or even increases 1 h after performing 0.2 or 0.12% CHX mouthwash (Fernandes-Naglik *et al.*, 2001; Jenkins *et al.*, 1989).

According to the findings of these studies and the local side effects of chorhexidine thet have been reported such as brown discoloration of the teeth, some restoration and dorsum of the tonge and bad salt taste (Flotra *et al.*, 1971) can suggest that the usage of persica mouth wash had a comparable antibacterial effect to chlorhexidine.

CONCLUSION

The overall results of this clinical study supported the conclusion that 0.2% CHX and PM had both antimicrobial effects against *A. actinomycetemcomitans* in periodontally healthy individuals and in patients with chronic periodontitis at different time intervals from 30 sec up to 300 min after rinsing with mouthwashes but they were not statistically significant at different time intervals in each groups. This study suggests that PM had a greater antimicrobial effect up to 120 min while 0.2 CHX had less antimicrobial effect but had longer substantivity in periodontally healthy subjects.

REFERENCES

Al-Lafi, T. and H. Ababneh, 1995. The effect of the extract of the miswak (chewing sticks) used in Jordan and the Middle East on oral bacteria. Int. Dent. J., 45: 218-222.

Darmani, H., T. Nusayr and A.S. Al-Hiyasat, 2006. Effects of extracts of miswak and derum on proliferation of Balb/C 3T3 fibroblasts and viability of cariogenic bacteria. Int. J. Dent. Hyg., 4: 62-66.

Ezmirly, S.T., J.C. Cheng and S.R. Wilson, 1979. Saudi Arabian medicinal plants: Salvadora persica. Planta Med., 35: 191-192.

Fernandes-Naglik, L., J. Downes, P. Shirlaw, R. Wilson, S.J. Challacombe, G.K. Kemp and W.G. Wade, 2001. The clinical and microbiological effects of a novel acidified sodium chlorite mouthrinse on oral bacterial mucosal infections. Oral Dis., 7: 276-280.

Fischman, S.L., 2000. The history of Oral hygiene prouducts: How far have we come in 6000 years. Periodontology, 15: 7-14.

Flotra, L., P. Gjermo, G. Rolla and J. Waerhaug, 1971. Side effect of chlorhexidine outhwashes. Scand. J. Dent. Res., 79: 119-125.

Jajarm, H.H., A. Jahanbin, N. Mokhber, S. Gooyandeh, A. Mansourian and J.M. Beitollahi, 2009. Effects of Persica mouthwash on oral microbiota of cleft lip and palate patients during fixed orthodontic treatment. J. Applied Sci., 9: 1593-1596.

Jenkins, S., M. Addy and R. Newcombe, 1989. Comparison of two commercially available chlorhexidine mouthrinses: II. Effects on plaque reformation, gingivitis and tooth staining. Clin. Prev. Dent., 11: 12-16.

Khalessi, A.M., A.R. Pack, W.M. Thomson and G.R. Tompkins, 2004. An *in vivo* study of the plaque control efficacy of Persica: A commercially available herbal mouthwash containing extracts of *Salivadora persica*. Int. Dent. J., 54: 279-283.

Mortazavi, M. and N. Balali, 2001. Intra oral effect of persica mouthwash, irsha, poidone iodine 1%, chlohixidine and hypertonic chloride sodium on the rate of mutants streptococci of saliva. Sch. Dentistry Shiraz Univ. Med. Sci.,

Rao, D.B., A.M. Hegde and A.K. Munshi, 2001. Caries prevalence amongst handicapped children of South Canara district, Karnataka. J. Indian Soc. Pedod. Prev. Dent., 19: 67-73.

Salari, M.H. and Z. Kadkhoda, 2004. Rate of cultivable subgingival periodontopathologic bacteria in chronic periodontitis. J. Oral. Sci., 46: 157-161.

Salehi, P. and M.D. Sh, 2006. Comparison of the antimicrobial effects of persica mouthwash with chlorhexidine on streptococcus mutans in orthodontic patients. Disability Advocacy Res. Unit, 14: 178-182.