LEVELS OF INFECTION AND COLONIZATION OF SOME ORAL BACTERIA AFTER USE OF NAF, CHLORHEXIDINE AND A COMBINED CHLORHEXIDINE WITH NAF MOUTHRINSES

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SHORT COMMUNICATION

ABSTRACT

This study was carried out to evaluate levels of infection of bacteria involved in caries and oral streptococci and their colonization after use of mouthrinses with NaF, chlorhexidine and chlorhexidine plus NaF. Combination of chlorhexidine and fluoride was the only agent that reduced levels of *Streptococcus mutans* (group) for 30 days.

Key words: chlorhexidine, sodium fluoride, cariogenic bacteria, oral streptococci

Mechanical methods are known for prevention and control of oral diseases. Some authors consider that they consume time for both professional and patients (14). It is important to consider yet that they are laborious and difficult for many patients (14,24) and impossible to others (24). Based upon these considerations and aiming to make dental plaque control more accessible to a significant proportion of population less laborious methods are wished (32). Among these methods are the chemical controls that have fascinated men for over 400 years (7). Chlorhexidine was originally introduced as commercial products in 1957 in Europe as an antiseptic cream for skin wounds (18). It has generated an increasing interest among dental community since its introduction as a 0.2% mouthrinse in experimental gingivitis study in 1970 (16). Apparently by acting via a generalized perturbation of the bacterial membrane rather than the antibiotic method, there is much less opportunity for bacterial resistance development, which was an early concern with it (18). Concerning fluoride, the first mouthrinse recommended in the United States was in the fourties for schoolchildren as caries prevention. Nowadays, FDA authorized the sale under control with mouthrinses of 0.05% sodium fluoride (NaF) (10). In spite of several antibacterial mechanisms suggested for the action of fluoride, it seems that the effect

over plaque activity is low, reducing only its metabolic activity (2,9) but not reducing bacterial concentration (17). Therefore, the purpose of this study was to evaluate levels of infection of major bacteria involved in caries process and the major group of bacteria isolated in the mouth, the streptococci, and their colonization after use of mouthrinses with NaF, chlorhexidine and chlorexidine plus NaF.

The subject population was selected from a group of military cadets from 18 through 25 years of age by means of previous screening based on levels of infection of *mutans* streptococci (>250.000 CFU / ml saliva) and therefore submitted to a high microbiological risk of caries (30). All the 60 selected patients signed an agreement term where they became aware of aims, risks and benefits of the study, allowing their inclusion. The subject population was divided into three groups according to products that they would use. This study was a double-blind where each group had 20 subjects. Subjects rinsed twice a day during 15 days with 10 ml of only one of the following solutions: a) NaF (0.05%, aqueous commercial solution prepared by Herpo, Rio de Janeiro, RJ) b) 0.12% of chlorhexidine digluconate (Periogard®, Colgate-Palmolive, Osasco, SP), c) 0.05% of NaF plus 0.12% of chlorhexidine digluconate (Duplak®, Herpo Ltda, Rio de Janeiro, RJ) according to recommendations for patients with

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high risk of caries (15). Thirty minutes after toothbrushing, the individuals rinsed with solutions containing chlorhexidine to avoid interference of sodium lauryl sulfate from dentifrices (1). When the period of 15 days was over, the patients remained under observation for a month for disclosing concentration of bacterial population studied. To verify levels of infection and colonization by *mutans* streptococci, lactobacilli and total oral streptococci, saliva stimulated with gum base was collected from all patients in three periods: a) before treatment with each product (baseline), b) immediately after finishing treatment (T_0), c)15 days after (T_{15}), d) 30 days after (T_{30}). Saliva was collected and transported in a cooler, and was immediately processed. Concerning total oral streptococci, diluted saliva was inoculated on Mitis-Salivarius Agar (Difco Laboratories, Detroit, Michigan) (3). For cultivation of *mutans* streptococci diluted saliva was inoculated on Mitis-Salivarius Agar added with 15% of sucrose, 0.1% potassium tellurite solution and bacitracin (0.2 IU/ml) (6). These plates were incubated in anaerobic atmosphere for 48 h and after this period colonies were counted. Concerning oral lactobacilli, diluted saliva was inoculated with pour-plate technique on Rogosa-SL Agar (Difco Laboratories, Detroit, Michigan) (25) and incubated in aerobic atmosphere at 37ºC for 72h. The same procedure used for other bacteria was employed for counting these microorganisms. Data obtained were statistically analyzed for differences within and among groups and periods of experiment into each groups by means of the ANOVA and Tukey's tests. The significance level was established at 5% ($p < 0.05$).

Considering all subject groups in relation to oral streptococci, in Table 1 is observed that there was no significant difference among them ($p < 0.05$) nor among period of experiment within each group ($p < 0.05$). According Table 1, subjects that mouthrinsed with NaF (group 1) showed no reduction of their levels of infection by *mutans* streptococci alongside the study with no significant difference among periods of experiment ($p < 0.05$). However, concerning those subjects who mouthrinsed chlorhexidine combined with NaF (group 2), there was a significant difference among experimental period ($p > 0.05$). This difference was observed among values of baseline and at different times (T_0, T_{15} and T_{30},). Concerning individuals from group 3, who mouthrinsed with

chorhexidine, levels of infection by *mutans* streptococci were significantly reduced ($p > 0.05$) between baseline and T_0. Comparing to other periods with baseline we can observe that there is no significant difference ($p < 0.05$). Comparing levels of infection by *mutans* streptococci with periods among groups no significant difference was observed ($p < 0.05$). This shows that the reduction observed in group 2 (T_0, T_{15} and T_{30}) and, that observed in group 3 (T_0) was not sufficient to reduce levels below those observed in group 1 at the same period. Considering all groups, in Table 1 is observed with relation to lactobacilli there was not a significant difference among them ($p < 0.05$) nor among periods for each group ($p < 0.05$).

Recently, the association of chlorhexidine and fluoride was proposed. Thus, this study was carried out to evaluate levels of infection and colonization of the major cariogenic bacteria and oral streptococci after use of fluoride, chlorhexidine and fluoride plus chlorhexidine mouthrinses. After two weeks of mouthrinses of fluoride no reduction was observed on the levels of infection by oral streptococci, *mutans* streptococci and oral lactobacilli nor any difference among baseline, at T_0, T_{15} and T_{30} of this treatment group regarding the other groups. Concerning fluoride it was reported that minimal inhibitory concentration to viridans streptococci was 0.125% (23), therefore a concentration higher than our study (0.05%). Major properties of fluoride in plaque control and as an antimicrobial product are the abilities to change the enamel surface energy (28) and inhibit the glycolysis and transport of nutrients based on phosphoenolpyruvate (8,17) and in a system based on ATPase linked to cell membrane by pH gradient (9). Some oral lactobacilli are ten times more resistant to fluoride than streptococci (8,17,22). In our study the reduction of lactobacilli was not observed due to low concentration used in daily mouthrinses. Combination of fluoride and chlorhexidine was effective to reduce levels of *mutans* streptococci (around 1 log.) and to keep it reduced for 30 days. However no reduction was observed with oral streptococci and lactobacilli. Our results are in accordance with most the authors where is observed a broad effect of this association to reduce *mutans* streptococci (12,20,29,30). This effect is formerly derived from bactericidal

Table 1. Means ± standard-deviations (in LOG) of number of CFUs of oral streptococci, *Streptococcus mutans* (group) and lactobacilli / ml of saliva in several periods (in days) and groups of patients treated with NaF, NaF + Chlorhexidine (CHX) and CHX.

	Oral streptococci			*Streptococcus mutans* (group)			Lactobacilli		
Periods	NaF	NaF+CHX	CHX	NaF	NaF+CHX	CHX	NaF	NaF+CHX	CHX
Baseline	7.87 ± 0.66	7.86 ± 0.71	7.85 ± 0.50	5.86 ± 0.69	6.10 ± 0.65	5.86 ± 0,68	3.82 ± 1.10	4.36 ± 1,91	4.78 ± 1.54
T_0	7.95 ± 0.45	7.63 ± 0.66	8.06 ± 0.62	5.60 ± 0.70	5.30 ± 0.72*	5.20 ± 0,55*	4.11 ± 1.38	3.88 ± 1,96	4.00 ± 1.57
T_{15}	7.83 ± 0.52	7.78 ± 0.64	8.01 ± 0.48	5.43 ± 0.74	5.48 ± 0.66*	5.36 ± 0,99	4.26 ± 1.36	4.06 ± 1,69	4.23 ± 1.34
T_{30}	7.97 ± 0.43	7.94 ± 0.55	7.99 ± 0.59	5.58 ± 0.72	5.45 ± 0.90*	5.34 ± 0,80	4.10 ± 1.77	3.56 ± 1,99	3.85 ± 1.59

* values different significantly ($p > 0,05$); baseline = before treatment; T0 = immediately after treatment; T15 = 15 days after treatment; T30 = 30 days after treatment.

action of chlorhexidine (19,20) followed its bacteriostatic action that in association with fluoride would inhibit bacterial metabolism in different sites. Bacterial recolonization is observed after some days (26,29) or months based on adopted treatment, infection levels, sucrose ingestion or dental restorations. In this study the recolonization of *mutans* streptococci did not occur 30 days after treatment, probably due to combined action of both products and because infection level at baseline was high. Despite reduction of *mutans* streptococci levels, oral streptococci were not total, suggesting that the site and function of *mutans* streptococci in oral ecosystem may be occupied by other bacteria, probably some strain of streptococci not reduced by this treatment. This hypothesis is supported by the fact that some oral streptococci strains show variable susceptibility to chlorhexidine and fluoride (17,19,21). Concerning oral lactobacilli, levels were not reduced by fluoride and chlorhexidine probably because these microorganisms were hidden in habitats not affected by these products as observed among irradiated patients (12). Daily mouthrinses with chlorhexidine alone reduced levels of infection by *mutans* streptococci immediately after treatment (T_0), afterwards they returned to baseline levels. Despite of some other studies had observed that this reduction had been more prolonged (4,5,11), in our study this fact was not observed probably due to low concentration used. Toxic effects of chlorhexidine on *mutans* streptococci is due to affect the hydrofobicity (13). Oral streptococci and lactobacilli were not affected by treatment with chlorhexidine mouthrinses. Oral streptococci shows varied susceptibilities to chlorhexidine (19,21) from 4 to 32 µg / ml (27). Oral lactobacilli frequently are not affected by chlorhexidine because of difficulty to achieve niches of lactobacilli into mouth (4,17). Regardless of reduction promoted by the use of chlorhexidine with fluoride for 30 days after mouthrinses and chlorhexidine immediately after treatment, these reductions must be questioned. Indeed infection levels observed after those rinses did not reduce as significantly as in fluoride group. In addition those solutions probably causes shifts in oral microbial ecosystem, thus, we can suggest that these compounds can not be used without prescriptions and should be specifically indicated for some patients. On the other hand, we observed that the combination of chlorhexidine and fluoride solution is able to reduce *mutans* streptococci by 30 days. Finally, we concluded that the use of this solution, if not well controlled, could be selective for other streptococci in oral environment.

ACKNOWLEDGMENTS

RESUMO

Níveis de infecção e colonização de algumas bactérias orais após o uso de soluções de fluoreto de sódio, clorexidina e de uma combinação de fluoreto de sódio e clorexidina

O objetivo deste estudo foi avaliar os níveis de infecção de bactérias cariogênicas e estreptococos orais e sua colonização após uso de bochechos com NaF, clorexidina e clorexidina e NaF associados. Associação de clorexidina e NaF foi o único agente que reduziu os níveis de *Streptococcus mutans* (grupo) por 30 dias.

Palavras-chave: clorexidina, fluoreto de sódio, bactérias cariogênicas, estreptococos orais.

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