

Oral antibacterial effect of chlorhexidine treatments and professional prophylaxis in children

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Abstract

Aim: The primary aim of this longitudinal study was to evaluate additional effects of 4-week chlorhexidine digluconate (CHX) gel treatments to control *Aggregatibacter actinomycetemcomitans* counts in children after professional dental prophylaxis. *Porphyromonas gingivalis* and *Streptococcus mutans* counts were also determined to evaluate the secondary effects of anti-plaque treatments on microbial shifts. **Methods:** Twenty-six children with *A. actinomycetemcomitans* counts $\geq 4 \log_{10}$ /mL of saliva and/or Quigley-Hein plaque index ≥ 3.0 were enrolled in this study. Patients were randomly assigned to groups GI (placebo gel), GII (0.5% CHX gel), GIII (1% CHX gel), and GIV (2% CHX gel). Four sessions of treatment were performed during 4 weeks after a session of professional dental prophylaxis. Real-Time polymerase chain reaction (PCR) was used to determine viable microorganism counts in non-stimulated whole saliva samples collected at baseline, one week, one month and three months after interruption of treatments. **Results:** A reduction of all bacterial counts was detected after the 3-month follow-up in all groups. Lower counts of *P. gingivalis* were achieved from 1 week on after treatments. The 2% CHX concentration seemed to contribute to lower *A. actinomycetemcomitans* levels and increase *S. mutans* levels. **Conclusions:** Professional dental prophylaxis was effective to control salivary levels of *A. actinomycetemcomitans*, *P. gingivalis* and *S. mutans*. Additional antimicrobial effects, however, were not observed by the combination of professional dental prophylaxis and 4-week chlorhexidine gel treatments.

Keywords: aggressive periodontitis, professional prophylaxis, chlorhexidine, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Streptococcus mutans*.

Introduction

Aggressive periodontitis (AgP) affects younger individuals and is characterized by a rapid loss of the supporting connective tissue and alveolar bone around teeth in the presence of oral biofilm¹. The prevalence of AgP is higher among Black individuals than among Caucasian individuals, ranging from 0.1% to 7.6%, with a tendency for familiar clustering of cases².

Increased detection levels of *Aggregatibacter actinomycetemcomitans* in the oral cavity is an indicator for the risk of development of AgP in children, adolescents and young adults³⁻⁶. *A. actinomycetemcomitans* is a Gram-negative facultative anaerobic microorganism acquired via maternal transmission⁷⁻⁸. It plays an important role in the onset of localized AgP because of its virulence factors, such as RTX leukotoxin and cytolethal distending toxin, which trigger

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immunological host responses activating osteoclastogenesis and leading to tissue breakdown⁹.

Antimicrobial chemotherapeutic methods can be used to prevent AgP. Chlorhexidine digluconate (CHX) is effective against a large spectrum of Gram-positive and Gram-negative oral microorganisms¹⁰. Short-term adjunctive use of CHX gel and/or mouthrinses decreases periodontopathogen counts¹¹⁻¹² and limits supra/subgingival biofilm re-growth after mechanical removal of dental plaque¹³⁻¹⁶. Microbial recolonization of tooth surfaces, however, begins soon after antimicrobial treatments¹⁷, which might induce shifts in the composition of biofilms. Simultaneous identification of certain bacteria may also change the clinical severity of periodontal diseases. Ramsey and Whiteley¹⁸ (2009) demonstrated an enhanced resistance and virulence of *A. actinomycetemcomitans* in the presence of oral streptococci. De Soete et al.¹⁹ (2005) observed higher *Streptococcus mutans* counts in saliva of periodontal patients after scaling root planning (SRP). Mayorga-Fayad et al.²⁰ (2007) reported more prevalent detection of *Porphyromonas gingivalis* in AgP patients.

To the best of our knowledge, salivary microbial shifts after the association between mechanical removal of dental plaque and CHX gel treatments have not yet been investigated. The primary aim of this longitudinal study was to evaluate additional effects of 4-week CHX gel treatments to control *A. actinomycetemcomitans* counts in children after professional dental prophylaxis. *P. gingivalis* and *S. mutans* counts were also determined to evaluate the secondary effects of anti-plaque treatments on microbial shifts.

Material and methods

Participants

The present study was conducted after approval by the Ethics Committee of Bauru School of Dentistry, University of São Paulo (Process #47/2006). Parents of patients attending the Clinic of Pediatric Dentistry were invited to enroll their children in the study. Parental agreement was documented by signing a written informed consent.

In a preliminary screening, forty-eight 7-12-year-old children donated saliva and were examined by a calibrated dentist (intraobserver concordance $8 > 0.9$) to determine Löe and Silness²¹ (1963) gingival index (G-index) and Quigley & Hein modified by Turesky dental plaque index²² (DP-index). Patients with *A. actinomycetemcomitans* counts ≥ 4 log₁₀/mL of saliva and/or DP-index ≥ 3.0 could be included in the study. Consumption of antibiotics and non-steroidal anti-inflammatory drugs up to 30 days before the study, presence of supragingival calculus, severe malocclusion, oral lesions in soft tissues and use of orthodontic appliances were considered as exclusion criteria.

Twenty-six children were enrolled in the study and randomly assigned to groups GI (control, placebo gel), GII (0.5% chlorhexidine gel), GIII (1% chlorhexidine gel), and GIV (2% chlorhexidine gel) by Microsoft Excel 2003[®] software.

Clinical procedures

Non-stimulated whole saliva was collected into sterile glass beaker for 5 min. Saliva samples were stored at -20°C until DNA extraction of specific microorganisms²³. Professional dental prophylaxis with sodium bicarbonate jet was performed before the first session of treatment. For that purpose, dental plaque was previously disclosed by a solution of 0.2% fuchsine to ensure the quality of procedures. Then, CHX gels were dispensed into disposable trays and applied on teeth for 1 min. Formulations and chemical properties of CHX gels are depicted in Table 1. Excesses of gel were removed and children were instructed to restrict liquid and food intake for next 30 min. Treatments were conducted once a week for 28 days. Clinical appointments were scheduled 1 week, 1 month and 3 months after the interruption of gel treatments to collect saliva samples and determine gingival and dental plaque indexes.

Table 1 – Formulations and chemical properties of different gels.

Gel formulations	
0%, 0.5%, 1% or 2% chlorhexidine concentrations	
2% hydroxyethylcellulose	
0.1% benzalkonium chloride 95 v/v	
0.18% methylparaben	
0.05% propylparaben	
0.05% menthol crystal	
7% ethyl alcohol 96 v/v	
0.5% polysorbate 20	
15% sorbitol	
100 mL of distilled water	
Chemical properties	
pH	5.8-6.5
viscosity	~ 300.000 cPs
specific density	1.0546 g/mL

Real Time PCR tests

Real time PCR tests were performed to allow the detection and quantification of *A. actinomycetemcomitans*, *P. gingivalis*, and *S. mutans*. After defrosting and homogenizing the saliva samples, two aliquots of 500 µL saliva each were diluted in 1 mL of sterile demineralized water (1:2) and centrifuged (10,000 xg) at 4°C for 5 min. Supernatants were discarded and pellets were washed three times with sterile demineralized water (10000 xg, 4°C, 5 min)²³. One bacterial pellet was incubated with 100 µL of a solution for cellular lyses (20 U/mL mutanolysin plus 0.2 mg/mL lysozyme) at 37°C for 2 h. This mixture was boiled (100°C, 10 min) to extract bacterial DNA from *S. mutans* cells. Other bacterial pellet was used to extract DNA from *A. actinomycetemcomitans* and *P. gingivalis* cells. The pellet was suspended in a mixture of 100 µL DNase-free water and 100 µL InstaGene Matrix (Bio-Rad Laboratories Inc., Hercules, CA, USA), and incubated at 56°C for 30 min. Then, the samples were vortexed and boiled for 10 min. Unbroken cells and large debris were removed by subsequent centrifugation

(10,000 xg, 3 min). Supernatants were used for Real-time PCR DNA analysis.

Real-Time PCR DNA analysis was performed in an ABI Prism 7000 system (Applied Biosystems, Warrington, UK), 1X Universal PCR Master-Mix (ABI), 200 nM of specific primers, 1µL of the supernatant, and 250 nM of each quencher dye (6-carboxyfluorescein and 6-carboxytetramethylrhodamine). The specificities of the primers were initially confirmed by BLAST with the National Center for Biotechnology Information server (<http://www.ncbi.nlm.nih.gov>). Oligonucleotide primer sequences are shown in Table 2. Bacterial DNA levels were determined using the Ct method and normalized by the volume of the supernatants.

Table 2 – Sequences of oligonucleotide primers.

	Sequence (5' – 3')
A. actinomycetemcomitans	
Forward	CAGCATCTGCGATCCCTGTA
Reverse	GCCCTTTGTCTTTCCTAGGT
P. gingivalis	
Forward	TACCCATCGTCGCCTTGGT
Reverse	CGGACTAAAACCGCATACACTTG
S. mutans	
Forward	GCCTACAGCTCAGAGATGCTATTCT
Reverse	GCCATACACCACTCATGAATTGA

Statistical analysis

Data were analyzed by NCSS/PASS® 2000 statistical software (Kaysville, UT, USA). Microorganism counts were log-transformed before statistical tests. Normality and homogeneity of data were respectively evaluated by Kolmogorov-Smirnov and Levene tests. Statistical differences were assessed using parametric one-way ANOVA and Bonferroni post-hoc test. Correlations between gingival or dental plaque indexes and microorganism counts were observed by Pearson's test. The p values <0.05 were considered significant.

Results

Gingival and Dental Plaque Indexes

Fourteen male and 12 female children were enrolled in the study (8.9±1.2 years). Correlations between gingival and

dental plaque indexes and *A. actinomycetemcomitans* (G-Index r=-0.24, DP-Index r=-0.10), *P. gingivalis* (G-Index r=-0.03, DP-Index r=-0.10), and *S. mutans* (G-Index r=-0.14, DP-Index r=0.01) counts were not observed at baseline. Both clinical indexes were statistically similar in different treatment groups and times (Table 3).

Microorganism counts

P. gingivalis and *S. mutans* were identified in 61.5% and 96.2% of children, respectively, at baseline. Bacterial counts did not differ significantly between groups throughout the study (p>0.05). The effectiveness of treatments was demonstrated by statistical differences observed among bacterial counts in different follow-up times. Considering all groups (Table 4), the mean of *A. actinomycetemcomitans* counts at 3 months (4.20±3.90) was significantly lower than at baseline (5.98±1.96) (p<0.05). Similar differences were verified in *S. mutans* counts between 3-month follow-up (3.14±2.67) and baseline (4.29±2.31) (p<0.05). *P. gingivalis* counts were statistically reduced from 1 week (1.73±2.74) after interruption of treatments compared with baseline (5.18±4.72) (p<0.05). Although *P. gingivalis* counts gradually increased to 3.21±3.42 at 1-month and 3.37±3.12 at 3-month follow-ups, the levels still remained significantly lower than at baseline (p<0.05).

The maintenance of lower *P. gingivalis* and *S. mutans* counts were noticed in 100% and 84.6% children, respectively, with initial *P. gingivalis* and *S. mutans* counts ≥4 log10/mL of saliva. On the other hand, respectively 54.5% and 69.2% children with initial *P. gingivalis* and *S. mutans* counts <4 log10/mL of saliva showed higher microorganism counts after 3 months of follow-up (Figures 1A, B and C).

A reduction in the percentage of children with salivary *A. actinomycetemcomitans* counts ≥4 log10/mL of saliva was observed in all groups since CHX treatments. The highest CHX concentration (2%) seemed to contribute to obtain lower levels of *A. actinomycetemcomitans* in most patients at the end of the study (Figure 1A).

In comparison with baseline, the percentage of patients with *P. gingivalis* counts ≥4 log10/mL of saliva decreased after 1 week and 1 month of placebo and 0.5% CHX gel treatments, whereas the percentage of patients did not vary after 1% and 2% CHX gel treatments. After 3 months, different

Table 3 – Mean (±SD) of gingival index (G-Index) and dental plaque index (DP-Index) of groups GI (placebo gel), GII (0.5% CHX gel), GIII (1% CHX gel), and GIV (2% CHX gel) after different follow-up times.

		Baseline	1 week	1 month	3 months
GI	G-Index	1.12±0.31	1.05±0.37	0.97±0.32	1.09±0.24
	DP-Index	3.07±0.50	3.30±0.50	3.51±0.43	2.75±0.95
GII	G-Index	0.84±0.54	0.84±0.42	1.00±0.34	1.12±0.12
	DP-Index	2.90±0.59	3.25±0.56	3.16±0.60	3.41±0.83
GIII	G-Index	1.15±0.30	1.04±0.25	1.07±0.36	1.02±0.19
	DP-Index	3.03±0.44	3.39±0.27	3.41±0.64	3.05±0.94
GIV	G-Index	0.88±0.53	1.06±0.30	1.25±0.29	1.05±0.46
	DP-Index	3.29±0.78	3.52±0.54	3.33±0.72	3.45±1.09

Table 4 – Mean (\pm SD) of salivary counts of *A. actinomycetemcomitans*, *P. gingivalis*, and *S. mutans* (log₁₀) at baseline, 1 week and 3 months after treatments with placebo gel (GI), 0.5% CHX gel (GII), 1% CHX gel (GIII), and 2% CHX gel (GIV). *ND* means microorganism not detected.

Treatment group	Patient	<i>A. actinomycetemcomitans</i>				<i>P. gingivalis</i>				<i>S. mutans</i>			
		Baseline	1 week	1 m	3 m	Baseline	1 week	1 m	3 m	Baseline	1 week	1 m	3 m
GI	1	8.94	10.44	7.36	5.23	10.12	8.97	3.79	4.49	9.45	8.07	7.30	7.15
	2	8.81	9.27	5.29	9.86	8.07	2.28	ND	4.60	9.10	ND	6.11	10.03
	3	5.13	2.06	2.29	ND	2.96	ND	ND	4.45	3.29	2.56	3.21	ND
	4	8.47	6.72	9.25	10.57	9.30	ND	8.55	2.89	8.10	6.44	1.56	ND
	5	4.88	3.78	3.19	2.43	ND	ND	ND	ND	2.51	4.08	0.54	ND
	6	3.02	3.47	ND	ND	ND	ND	ND	ND	5.23	5.09	5.28	ND
	7	3.34	2.87	ND	ND	ND	ND	ND	ND	5.06	7.21	5.19	5.22
	Mean\pmSD	6.1\pm2.6	5.5\pm3.3	3.9\pm3.6	4.0\pm4.6	4.4\pm4.7	1.6\pm3.4	1.8\pm3.3	2.3\pm2.3	6.1\pm2.8	4.8\pm2.8	4.2\pm2.5	3.2\pm4.2
GII	8	4.22	ND	3.23	2.44	6.91	ND	ND	ND	5.17	6.56	3.96	ND
	9	6.47	6.54	8.00	5.51	8.14	2.51	5.87	5.37	5.02	5.62	4.05	ND
	10	5.90	ND	7.85	5.98	9.60	2.30	3.21	5.47	5.50	ND	4.42	3.80
	11	5.55	5.84	6.81	2.87	7.25	ND	4.04	4.56	6.02	5.86	3.03	4.00
	12	8.15	6.46	5.91	2.89	6.64	ND	2.95	3.82	1.53	7.78	6.28	4.71
	13	4.58	6.74	ND	ND	5.89	1.71	ND	ND	1.91	ND	3.18	3.64
	Mean\pmSD	5.8\pm1.4	4.3\pm3.3	5.3\pm3.1	3.3\pm2.2	7.4\pm1.3	1.1\pm1.2	2.7\pm2.3	3.2\pm2.6	4.2\pm1.9	4.3\pm3.4	4.2\pm1.2	2.7\pm2.1
GIII	14	8.21	6.64	9.52	10.07	13.94	ND	10.11	11.11	5.42	ND	5.12	4.24
	15	5.43	6.65	6.87	11.78	11.74	3.99	3.33	ND	4.27	4.45	4.39	ND
	16	6.91	6.08	5.89	5.36	6.02	ND	ND	4.09	ND	5.54	ND	3.53
	17	6.45	3.76	ND	ND	13.14	2.70	5.64	6.09	5.61	4.40	3.48	4.18
	18	4.33	10.68	6.09	5.72	ND	7.30	4.86	5.83	3.07	2.36	6.76	5.79
	19	6.33	9.01	5.50	5.04	ND	6.30	ND	4.98	2.58	ND	2.72	3.84
	20	5.35	4.30	8.82	8.87	ND	ND	7.76	7.03	2.87	ND	2.12	ND
	Mean\pmSD	6.1\pm1.3	6.7\pm2.4	6.1\pm3.1	6.7\pm3.9	6.4\pm6.5	2.9\pm3.1	4.5\pm3.8	5.6\pm3.3	3.4\pm1.9	2.4\pm2.4	3.5\pm2.4	3.1\pm2.2
GIV	21	4.39	2.39	2.77	ND	ND	ND	3.00	ND	2.18	4.00	3.76	ND
	22	6.58	5.20	5.50	2.59	ND	ND	2.73	4.52	2.61	6.09	3.18	4.66
	23	6.77	9.28	8.94	ND	8.42	7.00	7.94	ND	3.50	4.65	2.94	5.13
	24	3.21	3.95	6.93	ND	ND	ND	ND	ND	2.33	2.83	4.96	3.40
	25	8.31	4.94	2.93	2.59	6.44	ND	ND	ND	6.16	2.44	0.78	4.15
	26	5.87	6.78	9.27	9.40	ND	ND	9.75	8.31	3.06	ND	3.11	4.10
		Mean\pmSD	5.9\pm1.8	5.4\pm2.4	6.1\pm2.8	2.4\pm3.6	2.5\pm3.9	1.2\pm2.9	3.9\pm4.1	2.1\pm3.5	3.3\pm1.5	3.3\pm2.1	3.1\pm1.4

CHX concentrations differed in controlling *P. gingivalis* levels. Higher percentage of children with *P. gingivalis* counts ≥ 4 log₁₀ was noticed in the group GIII as compared to groups GII and GIV (Figure 1B).

The results supported a trend of simultaneous increment of CHX gel concentrations and *S. mutans* counts along time. Treatments with 2% CHX gel increased *S. mutans* levels in most patients 1 week and 3 months after CHX treatments (Figure 1C).

Discussion

A positive impact on the prevention of AgP could be achieved by a short-term reduction of salivary periodontopathogens. This preliminary study demonstrated that one session of professional dental prophylaxis can be effective to reduce salivary counts of *A. actinomycetemcomitans*, *P. gingivalis* and *S. mutans* in children. Additional antimicrobial effects of CHX gels could not be demonstrated. After a 3-month follow-up, most children showed lower salivary counts of at least one microorganism, especially in patients with specific bacterial counts ≥ 4 log₁₀ at baseline.

The amounts of microbial deposits are inconsistent with the severity of periodontal tissue destruction in AgP patients²³. High proportions of *A. actinomycetemcomitans* and *P. gingivalis* can be associated with the onset of this disease. The rapid identification and quantification of periodontal microorganisms, therefore, permits early treatments to prevent the colonization of tooth surfaces for the maintenance of homeostasis of the periodontium. Currently, it is possible to monitor oral microbial compositions using diagnostic tools as Real-Time PCR²⁴⁻²⁵. Umeda et al.²³ (1998) demonstrated that PCR is a specific and highly sensitive method for detection of periodontopathogens in whole saliva. Our study focused on the potential application of CHX gels to reduce salivary pathogens, which would be able to colonize tooth and radicular surfaces, and increase the risk of developing periodontal diseases.

In the present study, disposable trays were used to deliver CHX gels in the oral cavity of children. Dental plaque and gingival indexes of all groups did not differ significantly after treatments. Francis et al.¹⁴ (1987) demonstrated efficient inhibition of dental plaque and control of gingival bleeding

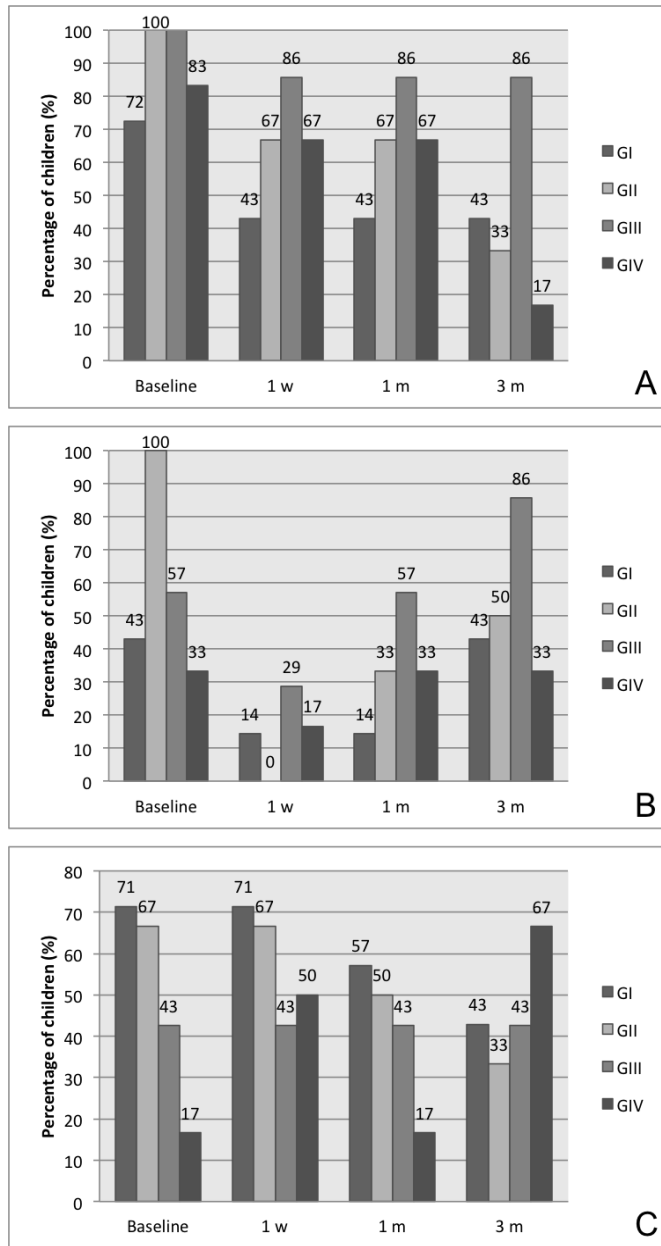


Fig. 1 – Percentage of children with *A. actinomycetemcomitans* (A), *P. gingivalis* (B) and *S. mutans* (C) counts ≥ 4 log₁₀/mL of saliva in different groups and follow-up times.

in patients treated with 4-week tray application of 1% CHX gel. Slot et al.¹⁶ (2010) reported an improvement of dental plaque control after tray application of 1% CHX gel compared to 0.12% CHX dentifrice gel. Our contrasting results could be explained by the inclusion of healthy periodontal patients with low scores of dental plaque and gingival indexes at baseline. According to Berchier et al.²⁶ (2010), the increase of CHX concentration seems to enhance the dental plaque control, although this could not be considered clinically relevant.

Since the diversity of microorganisms from saliva and dental plaque has been estimated in about 19,000 species-level phylotypes²⁷, multiple microbial clusters are possible among individuals. In our study, the host-specific microbial

load can be illustrated by the detection of *P. gingivalis* and *S. mutans* in respectively 61.5% and 73% children at baseline. Similar treatments resulted in diverse antimicrobial responses in different patients: 4-week 1% CHX gel treatment was effective to reduce all bacterial counts in patient #17, whereas the same regimen increased all microorganism counts in patient #18. Lower *S. mutans* and *P. gingivalis* counts and higher *A. actinomycetemcomitans* were detected in patients #14 and #15. Filoche et al.²⁸ (2008) showed a host specificity of antimicrobial agents against *ex vivo* microorganisms.

After a 3-month follow-up, professional dental prophylaxis was effective to reduce at least one specific bacterial count in all patients of group GI. Periodic dental visits may also have contributed to the enhancement of dental plaque control in children. The application of 0.5% CHX gel decreased simultaneously *A. actinomycetemcomitans*, *P. gingivalis* and *S. mutans* counts in most patients. Higher counts of bacteria were detected in four out of seven patients treated with 1% CHX gel. Two percent CHX gel treatments were responsible for lowering *A. actinomycetemcomitans* counts in 83.3% patients and raising *S. mutans* counts in 66.7% patients at the end of the study. Although speculative, our findings could indicate a possible trend of microbial shifts between periodontopathogens and cariogenic microorganisms with increasing concentration of CHX gels. De Soete et al.¹⁹ (2005) reported a significant increase of detection of *S. mutans* after SRP. Clinical interventions could induce local shifts in composition of biofilms from predominant anaerobic and proteolytic microorganisms to aerobic and acidogenic microorganisms. Other microbial relationships can be expressed by association among specific substrate, microbiological composition and metabolism. For example, lactate - a metabolite of cariogenic bacteria - can be used as a carbon source for *Veillonella* sp. These bacteria are able to produce considerable amount of menaquinone, an organic source of vitamin K, essential to growth of *P. gingivalis* and *T. denticola*²⁹.

Thresholds of minimal *P. gingivalis* and *S. mutans* counts were not established in our inclusion criteria. Independently of treatment group, most patients with initial *S. mutans* and *P. gingivalis* counts ≥ 4 log₁₀/mL of saliva presented a trend to reduction of bacterial counts after 3-month follow-up, whereas respectively 69.2% and 54.5% patients with *S. mutans* and *P. gingivalis* counts < 4 log₁₀/mL of saliva presented a trend to increase bacterial counts along time. Moreover, faster reductions of *P. gingivalis* counts seemed to indicate more sensitivity to mechanical and chemotherapeutic treatments than *A. actinomycetemcomitans* and *S. mutans*. These findings suggest shifts in microbial compositions after treatments for dental plaque control. Sekino et al.¹² (2004) demonstrated that the combination of a 0.2% CHX mouthrinse and a 1% CHX gel decreased salivary microorganisms and blocked tooth recolonization by *A. actinomycetemcomitans* and *P. gingivalis*. A significant reduction of periodontopathogen counts after SRP and full-mouth disinfection with CHX was also described by Quirynen

et al.¹⁷ (1999). Perinetti et al.¹¹ (2004) achieved a reduction of *A. actinomycetemcomitans* and *S. mutans* counts in periodontal pockets after irrigation with 1% CHX gel.

Some issues should be discussed regarding the results. It is possible that significant differences were not observed among the different groups of this study due to some limitations: (1) small sample size, (2) allocation of participants into four distinct groups, and (3) diversity of antimicrobial responses from each patient. Also, inclusion criteria limited the enrollment of participants in this study, especially *A. actinomycetemcomitans* counts $\geq 4 \log_{10}/\text{mL}$ of saliva. Additionally, the follow-up could be extended to 6 months to adequately assess the effect of chlorhexidine substantivity on clinical and microbiological parameters.

In conclusion, this study demonstrated that mechanical dental plaque control can be used as an effective preventive strategy to control salivary counts of *A. actinomycetemcomitans*, *P. gingivalis* and *S. mutans*, contributing to the maintenance of periodontal health. Further randomized clinical trials are needed to establish safe protocols for demonstrating additional benefits of chlorhexidine treatments against pathogenic microorganisms in saliva, with minimum modifications of oral homeostasis.

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