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Original Article

Effect of Chlorhexidine Gel in Mentally Handicapped Children highly Infected with *Streptococcus Mutans*

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Abstract

Objective: To evaluate the effect of 1% chlorhexidine (CHX) gel on mentally handicapped children (n=11) highly infected with *Streptococcus mutans* (SM) and evaluate the re-colonization time of this bacterium after the treatment period. **Material and Methods:** Before CHX treatment, saliva samples were collected to establish baseline saliva SM levels. CHX gel was daily applied for 5 min, for 7 consecutive days. Saliva samples were then collected after 7, 30, 60, 90 and 120 days to evaluate SM oral re-colonization. Data were analyzed by the Wilcoxon test ($\alpha=0.05$). **Results:** When compared to baseline (5.7 / 3.0 x 10⁵), the collection performed 7 days after CHX treatment (0.06 / 0.2 x 10⁵) showed a significant reduction ($P < 0.05$) in SM saliva levels (mean / s.d., CFU ml⁻¹). In subsequent collections, a gradual SM re-colonization was observed, and after 60 days of treatment, the levels of this bacterium (0.8 / 0.9 x 10⁵) no longer statistically differed from SM levels considered high risk to caries (10⁵) ($P > 0.05$). **Conclusion:** Treatment with CHX gel for 7 days was effective in reducing the SM levels of mentally handicapped children and should be repeated after 60 days to control the risk of caries in these children.

Keywords: Chlorhexidine; *Streptococcus Mutans*; Saliva; Children.

Introduction

Streptococcus mutans (SM) are considered the most cariogenic microorganisms in dental biofilm due to their capacity to use dietary carbohydrates to synthesize extracellular polysaccharides and to their acidogenic and aciduric properties [1,2]. Salivary *S. mutans* levels have been used as an indicator of caries risk [3,4], being therefore a form of controlling cariogenic activity through the reduction of the oral levels of these bacteria by mechanically removing the dental biofilm [5]. However, efficient tooth brushing is a difficult task for mentally handicapped children due to their physical and mental limitations [6-8]. Consequently, the use of antimicrobials agents appears to be particularly suitable for these children [9].

Among the antimicrobial agents available for dental use, chlorhexidine (CHX) is the most effective and most widely documented substance in controlling cariogenic activity [1,10]. This is because this chemotherapeutic agent is very efficient in reducing the oral levels of some microorganisms, in particular SM, as this bacterium has exceptional sensitivity to this antimicrobial agent [11]. The major advantage of CHX over most other compounds lies on its high oral substantivity [13], due to capacity of this cationic substance to adhere to soft and hard tissues, as well as to bacterial cell walls, allowing the slow release of this antimicrobial agent to the oral cavity [12].

Given lack of coordination of mentally handicapped children, CHX gel administrated in individual trays may be a good regimen to control and prevent these patients from caries activity. Studies have shown that CHX gel treatment is very effective in reducing the SM levels of children highly infected by this bacterium, and these levels are gradually re-established over a period of 2-6 months after treatment [12-14]. However, no studies were found in literature evaluating the effect of this antimicrobial agent in mentally handicapped children highly infected with SM. Thus, the aims of this study were: (a) to evaluate the effect of 1% CHX gel in reducing the SM levels of mentally handicapped children; and (b) to evaluate the re-colonization time of the bacteria after CHX gel treatment.

Material and Methods

This study was a prospective, double-blind clinical study approved by the Ethics Committee of the University Hospital - Federal University of Maranhão (Protocol No. 33104-00320 / 2002).

Eleven children aged 6-10 years of both genders with moderate intellectual disability [15] regularly attended at the Association of Parents and Friends of Exceptional Children (APAE) in São Luís, Brazil were selected. Parents or guardians of these children read and signed the Informed Consent Form (ICF), which contained explanations about the research objectives, procedures to be performed and the possible risks and benefits from the participation in the survey.

The number of patients was determined by preliminary tests, which showed the sample size able to detect statistical differences (Wilcoxon test, $\alpha = 0.05$, 80% power; BIOSSTAT, version 5.0, 2007, Brazil). The inclusion criteria involved children of both genders, with high saliva SM levels.

None of the selected patients used antimicrobial or chemotherapeutic drugs systemically, mouthwash or products of topical use for a minimum of six months before the start of the experiment. All children received restorative dental care before the beginning of the experiment.

Treatment with of 1% CHX gel was conducted for a period of 7 days, one gel application every 24 h. Before CHX treatment, saliva samples were collected in order to establish the initial SM saliva levels (baseline). After the treatment period, saliva samples were collected at 7, 30, 60, 90 and 120 days after the last gel application so that the effect of CHX gel on SM levels could be observed, as well as the re-colonization time of this microorganism. Microbiological analysis of saliva was done using a specific test for SM.

Collection of saliva samples

Saliva collections were performed in the morning, between 09.30 a.m. and 11.00 a.m. at least 2 hours after breakfast and before the morning snack. Thus, a sample of about 2.0 ml of saliva was collected after each patient had chewed one stick of gum base. The saliva produced in the first 30 seconds was discarded, and collection started after that time. Sterile test tubes were used for saliva collection, containing ten glass beads to facilitate homogenization in tube shaker (Vortex). Before processing, the tubes were kept on crushed ice [16].

Microbiological analysis of saliva

To analyze the SM levels, CARITEST-SM kit was used (HERPO - Dental products LTDA, Rio de Janeiro, RJ, Brazil). In aseptic chamber, the bacitracin content was added to bottles containing buffered diluent, this procedure was carried out in a period of time less than 30 minutes before using the Caritest-SM. The test tubes containing the saliva samples were individually homogenized in vortex for 1 minute, then 1.5 ml aliquots were transferred to tubes containing the diluent and bacitracin mixture being submitted, again, to agitation for 30 seconds. Slides containing culture medium distributed on both sides were removed from their original containers and soaked in saliva-diluent mixture, remaining for a period of 30 seconds, during which contents of the CO₂-generating envelope was added to the empty flask. Then, slides were removed from the saliva-diluent mixture to remove excess liquid and transferred to their original bottles with the addition of 2 drops of sterilized distilled water to the CO₂-generating mixture, with airtight closing being carried out. The tubes in vertical position, were incubated at 37°C for 48 h and kept for 24 hours at room temperature before comparing the growth density on the surface of both sides of the culture medium with the evaluation table provided by the manufacturer. Only colonies with presumptive characteristics of belonging to the *mutans* group were considered; no biochemical test was performed to identify the species of the group.

A single investigator has undergone a prior training in order to provide the necessary conditions for the conversion of the appearance of the incubated blade in numbers corresponding to the growth densities (CFU ml⁻¹ of saliva). The scale with the population density of colony-forming

units, provided by the manufacturer, showed 6 levels of CFU classification: 10 000, 50 000, 100 000, 250 000, 500 000 and one million CFU ml⁻¹ saliva. In terms of microbiological count, children with SM levels higher than 10⁵ CFU ml⁻¹ saliva showed high SM infection, indicating a high risk of caries [3,4].

Application of 1% chlorhexidine gel

Before starting the experiment, children and parents or guardians received the same dental cleaning instructions and were told to reduce sucrose consumption of volunteers. After the first saliva collection (baseline), 1% CHX gel treatment was initiated (Farmácia Facial, São Luís, MA, Brazil), which was conducted for a period of seven days, with gel application for 5 min every 24 h. For gel application, disposable casts were used.

Statistical Analysis

The nonparametric Wilcoxon test was used for statistical analysis of results. Calculations were performed using the BIOSTAT software (version 5.0, 2007; Brazil), and significance level adopted was 5%.

Results

Treatment with 1% CHX gel was effective in reducing SM levels of mentally handicapped children (Figure 1), with a statistically significant difference between the initial levels of this microorganism and the levels observed in all samples collected after the application period ($P < 0.05$) (Table 1).

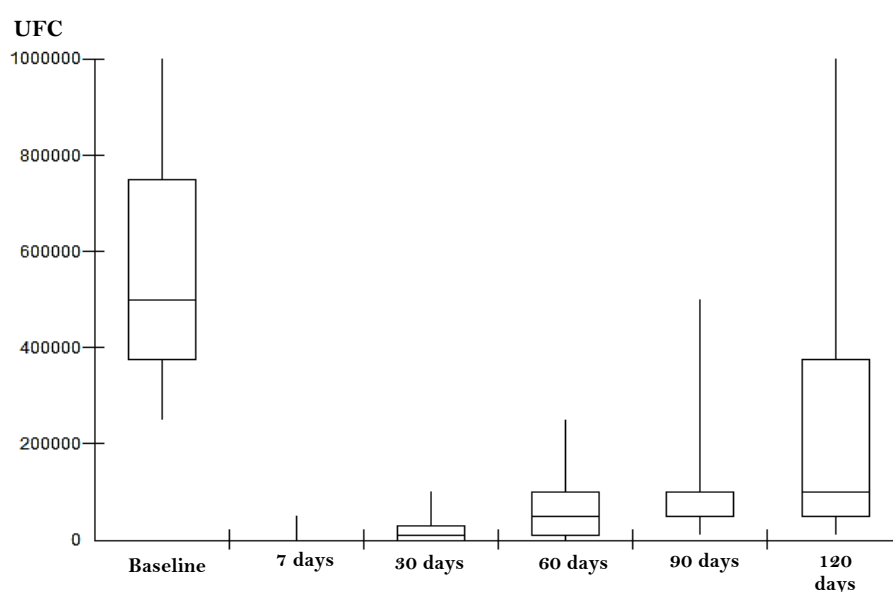


Figure 1. Box-Plot (Median and Quartiles) of SM levels in saliva (CFU ml⁻¹) before (baseline) and 7, 30, 60, 90 and 120 days after CHX gel application (n = 11).

Table 1. Mean and standard deviation (SD) of the SM levels in saliva (CFU ml⁻¹) before (baseline) and at 7, 30, 60, 90 and 120 days after application of 1% CHX gel (n = 11).

	Saliva collection periods					
	Baseline	7 days	30 days	60 days	90 days	120 days
Mean	5.7 x 10 ⁵	0.06 x 10 ⁵ *†	0.2 x 10 ⁵ *†	0.8 x 10 ⁵ *	1.2 x 10 ⁵ *	2.9 x 10 ⁵ *
S.D.	3.0 x 10 ⁵	0.2 x 10 ⁵	0.3 x 10 ⁵	0.9 x 10 ⁵	1.4 x 10 ⁵	3.8 x 10 ⁵

*Result with statistically significant differences from baseline (P < 0.05); †Result with statistically significant difference of the SM level considered high risk for dental caries (10⁵ CFU ml⁻¹ saliva) (P < 0.05).^{3,4}

Considering that children with SM levels higher than 10⁵ CFU ml⁻¹ saliva already have high infection by SM, indicating a high risk of caries.^{3,4} The present study compared this SM level with levels observed in collections carried out after treatment with 1% CHX gel (Table 1) and concluded that after 60 days of the application period, SM levels no longer differed from the level of this microorganism considered high risk for caries (P > 0.05).

Discussion

Among the methods available for CHX application, the form of gel applied by means of individual casts is preferable for mentally handicapped children, as the method decreases the risk of swallowing gel by volunteers. CHX in the form of gel is found in 1-5% concentration. Studies have shown that increasing CHX gel concentration from 1% to 5% does not increase its effect against SM [12] and most studies evaluating the use of the substance used concentration of 1% [12,17]. In this study, the application of 1% CHX gel for 7 consecutive days caused the SM levels to remain very low and, in some cases, undetectable. These results are in agreement with previous studies [18,19] but in these papers, CHX gel was applied for a period of 14 days. The results of this study are important, as they certify that 1% CHX gel may be used daily for a shorter period of time, without interfering in its effectiveness. In addition, adverse effects caused by CHX such as altered taste and burning sensation in soft tissues [17] commonly reported by patients when CHX in high concentration is applied for a long period of time, were not reported by mentally handicapped children treated in this study.

Although SM levels were reduced to low levels and even undetectable by CHX gel, as noted 7 days after gel application period, the treatment was not able to completely eliminate these bacteria in the oral cavity. The permanence of this microorganism in the oral cavity can be explained by the presence of reservoirs and / or retentive areas in the teeth slightly affected or not affected by the antimicrobial agent, from which SM start re-colonization after removal of the antimicrobial pressure [20]. In the other saliva collections performed after CHX gel treatment, it was observed a gradual reappearance of SM over time. However, 120 days after the treatment period, the levels of these microorganisms showed statistically significant differences from the initial levels (baseline). These results are in agreement with previous studies [13,14,21] which showed a gradual reappearance of

SM in a period of 180 days after the levels of this bacterium have been significantly reduced with CHX gel application.

Although in all collections performed after treatment with CHX gel, the SM levels significantly differed from the initial levels (baseline), and the results of this study suggest that treatment with CHX gel should be repeated every 60 days, taking into account that for most patients in this study, SM levels observed in this period no longer statistically differ from the level of this microorganism considered high risk for dental caries ($\geq 10^5$ CFU ml⁻¹ saliva) [3,4]. Periods of gel reapplication similar to the present study were established by other authors who evaluated the effect of CHX gel in children with no disabilities. For these patients, CHX gel applications must be repeated in a period ranging from 60 to 90 days [12,17].

The high standard deviation (SD) observed in this study is justified because the re-colonization time of SM was not the same for all children, that is, while most children already needed a new gel application after 60 days, some children still remained at low levels of this microorganism. This variation in the re-colonization pattern was also reported by others authors [13,14,16]. Moreover, for all mentally handicapped children in the present study, the reapplication of 1% CHX gel was not necessary before 60 days of treatment performed.

Several factors can influence the re-colonization time of SM in individuals treated with chlorhexidine gel, including the number of restored tooth surfaces and retaining sites, as well as the amount of sucrose consumed daily by each volunteer [12,16,17]. In order to limit the effect of these factors on the re-colonization time of SM, restorative treatment of all children was made before the start of the experiment, and children and parents or guardians were advised to reduce sucrose consumption during the experiment period. However, a limitation of this study was not assessing the consumption of carbohydrate during the experiment period, as well as not having assessed the DMF index of these children, since these factors could help understanding the variation in the re-colonization pattern of SM in mentally handicapped children.

Conclusion

The daily treatment with 1% CHX gel for 7 days was effective in reducing the levels of SM in mentally handicapped children; and, considering the re-colonization pattern of SM in these individuals, treatment with CHX gel should be repeated after 60 days to control the risk of caries in these children.

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