Scientific Article

The Effect of 1% Chlorhexidine Varnish and 40% Xylitol Solution on *Streptococcus Mutans* and Plaque Accumulation in Children

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Abstract: ***Purpose:*** *The purpose of this study was to determine the effect of the association of 1% chlorhexidine varnish (CHX) and 40% xylitol solution (XYL) on Streptococcus mutans (SM) counts and plaque indices in 2- to 5-year-olds.* ***Methods:*** *Sixty-eight children were selected with medium levels (1 x 10^3) to very high levels (>1 x 10^5) of SM in the saliva. Subjects were divided into 4 groups of 17 children each: (1) CHX; (2) CHX+XYL; (3) XYL; and(4) 0.05% sodium fluoride (F). An assessment of SM levels and plaque indices was done on all children at baseline, 15 days, and at 1, 3, and 6 months. SM levels were determined by the spatula method.* ***Results:*** *Although the reduction in SM counts in all groups was statistically significant, differences among groups were not observed, and the CHX and F groups seemed to show the greatest effect. Plaque reduction was observed in all groups, whereas statistically significant decreases among groups were not observed.* ***Conclusions:*** *One percent chlorhexidine varnish associated with 40% xylitol solution tested in the present study does not provide significant suppression of Streptococcus mutans counts and reduction of plaque accumulation at any follow-up time points. (Pediatr Dent 2011;33:484-90) Received March 2, 2010 / Last Revision August 5, 2010 / Accepted August 6, 2010*

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While studies have shown that the incidence of dental caries in young children has decreased overall in the past 20 years in some countries, there is growing evidence of oral health disparities in countries having a lower socioeconomic level. There are many countries and minority populations where the incidence is still high.[1] Moreover, according to the (**WHO**),[1] caries incidence in children is still at an alarming level around the world. Therefore, there is great need for the development of preventive, practical, and cost-effective strategies for dental caries to be globally controlled.

A number of studies have provided evidence that, although dental caries is dependent on different factors, the main causative agent is *Streptococcus mutans* (***SM***) colonization and consequent plaque accumulation on dental surfaces. The higher the *SM* level, the higher the plaque accumulation and the greater the risk of developing carious lesions.[2-5] Therefore, control of *SM* levels is an important target of caries prevention and control.[1] Among the possible strategies in *SM* control, chemotherapeutic treatment regimens have received much attention and have presented satisfactory results in promoting *SM* suppression, plaque reduction, and, consequently, caries prevention or incidence decrease.[6] Of the chemical agents used in dentistry, chlorhexidine (**CHX**) and xylitol (**XYL**) seem to offer strong antimicrobial activity. It is of interest that these agents have completely different modes of action.

CHX is an antimicrobial substance having the best effect against *SM*.[7] In high concentrations (40%, 33%, 25%, 20%, or 10% CHX in varnish), it has an immediate bactericidal action, as it penetrates the cellular wall and causes cytoplasm precipitation. In smaller concentrations (3% and 1% CHX in varnish and gel and 0.2% and 0.12% CHX in rinse), it has hydrophilic-hydrophobic properties and a bacteriostatic effect that interferes with membrane transport, allowing its light-weight molecules to infiltrate into the offending micro-organism.[8]

XYL is a sugar substitute usually used in foods, candies, and chewing gum. It cannot be metabolized by *SM*, so it promotes changes in *SM* metabolism by means of a strain-selective process: XYL-resistant strains proliferate with limited capacity to adhere to hard surfaces compared with XYL-sensitive strains, which decrease in number.[9-11] These changes in the *SM* population lead to caries control.

Studies have been conducted combining CHX and XYL and combining these agents with others with the aim of obtaining better results when compared with their individual use.[12-14] According to their characteristics, this pairing would be expected to suppress bacterial levels via CHX use, while less pathogenic strains would be suppressed via XYL use. Nevertheless, very few investigators have studied the association between these 2 substances; none of the studies was conducted in children, but only with adults,[15,16] the elderly,[17] or in vitro.[18]

CHX and XYL, separately or combined, seem to perform well against *SM* in these studies; however, these substances were never used in young children. Therefore, the purpose of this study was to determine the effect of the association of 1% CHX varnish and 40% XYL solution on *SM* counts and plaque indices in 2- to 5-year-olds.

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Methods

This study was approved by the Institutional Review Boards of Federal University of Rio de Janeiro, Rio de Janeiro, Brazil and University of Pittsburgh, Pittsburgh, Pa. Informed written consent was obtained from the children's legal guardians. The experimental design of the study is summarized in Figure 1.

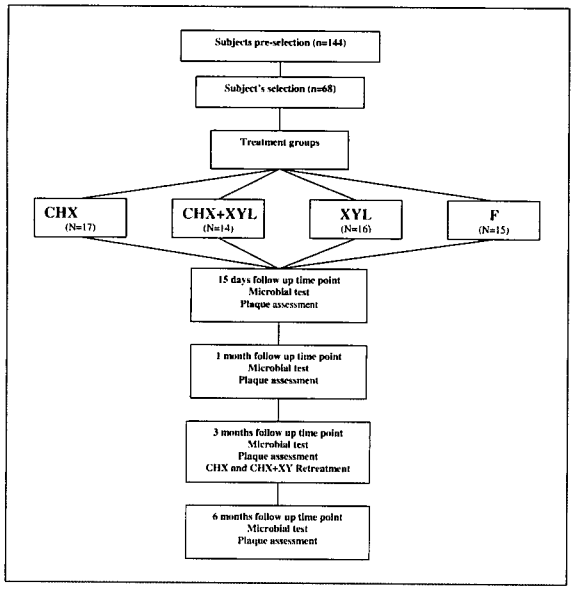

Figure 1. Study design.

Subject recruitment. This blinded investigation of 6-months duration recruited 2- to 5-year-old children from a public preschool and from a public pediatric dental clinic. A trained dentist examined all the children in a dental chair with standard light source and air jet, using a mouth mirror and dental explorer, after cleaning the children's teeth with a toothbrush to verify the condition of the dentition. Children with cavitated carious lesions were excluded, while those with active white spot lesions and restorations were allowed to continue in the study.

The mothers were interviewed by the same trained dentist about oral hygiene, dietary habits, fluoride usage, antimicrobial exposure of the child, and their knowledge about the chemical agents to be used in the study. During the interview, they received dietary and hygiene instructions for the child. Children who were taking systemic antimicrobial medication, systemic medication that could affect the oral microflora, or who had received oral application of any antimicrobial agent within 3 months prior to the study were excluded from the study. A total of 144 children were recruited to participate based on these criteria.

Pretest. Final selection from the intital 144 children was based on the subjects' salivary *SM* levels assessed by the tongue blade method.[19] In this technique, a 3-cm, sterile wooden spatula is used to collect saliva and inoculate the contact plate with the saliva. However, because of the small size of a 2- to 3-year-old's mouth, a 3-cm spatula would make this method not feasible in young children. In view of this, we developed a pretest to investigate the effect of the contact area of the tongue blades on *SM* counts in Rodac contact plates to verify the possibility of using a shorter spatula for young children.

SM 10449 (serotype c) was used for these experiments with batch cultures started from 90°C stocks. Primary cultures of *SM* (0.1 mL) were grown in 50-mL Trypticase soy broth supplemented with yeast extract in 5% carbon dioxide (CO_2) at 37°C overnight. Before sterilization, the media were treated with invertase to remove trace amounts of contaminating sucrose. Cultures were harvested by centrifugation and resuspended in an equal volume of fresh medium. Three different dilutions of *SM* were prepared:

1. a high concentration (1 x 10^5), representing a quantity of colony-forming units (CFU) higher than 101 (>101 CFU) in the plates;
2. a medium concentration (1 x 10^3), between 11 and 100 CFU (11-100 CFU); and
3. a low concentration (1 x 10^1), between 1 and 10 CFU (1-10 CFU).

For *SM* inoculation, 72 sterile wooden tongue blades, 1.8 cm wide, (1 for each Rodac plate) were used. The spatulas were rotated 10 times in the *SM* suspension; then, each side of the spatula was pressed against the *Mitis salivarius*, sorbitol, kanamycin, and bacitracin (**MSKB**) agar medium[20] onto Rodac plates of a predetermined length, inoculating the *SM*. Thirty-six 2.5-cm-long blades were used for group 1, and 36 3-cm-long blades were used for group 2. It is important to emphasize that each length represented different inoculation areas. All the plates were incubated in 5% CO_2 at 37°C for 48 hours. The resulting colonies were counted with the aid of a dissecting microscope on the spatula impression area by a previously trained observer.

SM counts were analyzed by 2-way analysis of variance (**ANOVA**) with Tukey-Kramer post-tests, with a significance level of 5%.

There were no statistically significant differences in colony counts across the plates ($P<.26$). Minor differences in the contact area of the spatula did not appear to influence the total *SM* counts under laboratory conditions. These results demonstrated the possibility of using a small area of spatula, if necessary, to collect and inoculate the saliva without influencing final results.

Subject's microbiological selection and plaque assessment. After the pretest results, the microbiological selection of subjects started. The spatula method was developed with the possibility of a small variation (2.5-3.0 cm) in the saliva area collection. For the microbial test, the parents were instructed to withhold their child's oral hygiene for 24 hours and not feed their child for 1 hour prior to the test.

Saliva samples were taken using a modified tongue blade method[19]: 2.5 to 3.0 cm of a sterile wooden spatula (18 cm 1.8 mm) was introduced into each child's mouth by the same dentist in a dental office and turned over 10 times to satisfactorily cover it with saliva. Excess saliva was removed during the withdrawal of the spatula by asking the child to close his or her lips. The 2 sides of the wooden spatula were pressed immediately onto a Rodac plate surface containing 12 mL of MSKB media.[20] Next, the plates were incubated for 72 hours in an anaerobic jar (BBL Gas Pak, Becton Dickinson, Cockeysville, Md) with 80% nitrogen gas (N_2), 10% hydrogen (H_2), and 10% CO_2 at 37°C. The period between saliva inoculation and incubation did not exceed 4 hours.

SM CFU were counted by the same trained dentist, in the spatula impression area with the aid of a stereoscopic microscope after 72 hours. Colonial morphology was used as the criterion for enumeration of *SM*: firmly adherent to the media and crystalline. *SM* CFU counts were expressed in scores according to the following criteria (modified from Weber's *SM* counts)[21]: 0=absence of *SM*; 1=low level (1-10 CFU=1 x 10^1); 2=medium

level (11-100 CFU=1 x 10^3); 3=high level (101-250 CFU=1 x 10^5); 4=very high level (>250 CFU >1 x 10^5).[21] Only children with moderate, high, and very high *SM* levels were selected for the study (N=68). The *SM* levels assessed for the subjects' selection was considered the baseline of *SM* levels.

At the same appointment, the plaque assessment was initiated. Plaque assessment of the labial surface of the primary maxillary incisors was performed without using disclosing solution. Each incisor received a score according to the presence and quantity of plaque: 0=absence of visible plaque; 1=visible plaque on less than half the surface; 2=visible plaque on more than a half the surface (modified from Weber's criteria for plaque scores).[21] This plaque assessment was considered the baseline plaque indices for the selected children.

Treatment. The 68 selected children were divided into 4 groups of 17 each, according to treatment: (1) 1% CHX varnish (Cervitec, Ivoclar Vivadent, Mississauga, Ontario, Canada); (2) 40% XYL solution; (3) CHX+XYL; and (4) 0.05% sodium fluoride (F) solution. A single trained dental hygienist performed all treatment procedures and provided all the information and instructions for the children and their parents in a dental office. The dentist was blind for the group allocation.

In the CHX group, the CHX varnish was applied by quadrant to all dental surfaces with a microbrush over a 3-month interval (every 3 months). Before application, the teeth were professionally cleaned, isolated with cotton rolls, then air-dried for 30 seconds. Parents received written and verbal instructions to not: give any food or drink for 1 hour after treatment; brush the child's teeth for 24 hours; floss for 1 week; and provide sweet or hard foods for the next 24 hours. In the XYL group, the mothers were instructed to apply the xylitol solution at home with gauze every night after the last oral hygiene throughout the entire study period (6 months). Each application was to expend 24 drops—6 drops for each hemiarch. Each child received a bottle of 100 mL XYL to take home. The XYL bottle was replaced every 3 months or when necessary. In the CHX+XYL group, mothers were instructed the same way as in the previous groups. In addition, XYL solution use was started 24 hours after CHX application. In the F group, mothers received the same instructions given in the XYL group. Toothbrushes were to be replaced by new ones at each retreatment appointment. The microbial test and plaque assessment were repeated after 15 days, 1 month, 3 months, and 6 months from the first treatment appointment (Figure 1).

Treatment acceptance. The Faces Scale[22] was used to measure the children's acceptance of treatment. This scale consists of 3 face pictures (Figure 2), which were shown to children older than 3-years-old to check their reaction. After the first treatment, the child was asked to point to the face that was similar to his or her impression of the treatment. Children younger than 3 years old were excluded from this evaluation because they could not understand the difference between satisfaction and dissatisfaction on the Faces Scale.

Statistical analysis. Analyses of the microbiological and clinical data were done using SPSS 11.0 software (SPSS Inc, Chicago, Ill). Nonparametric statistical analyses were performed using Kruskal-Wallis ANOVA. Values of $P<.05$ were considered significant.

Results

From the 68 selected and treated children, 62 [37 (~60%) boys and 25 (~40%) girls] returned to follow-up. The mean age was 3.89±0.89-years-old. At baseline, 39% of the subjects were colonized with *SM* at a medium level (score 2), 23% at a high level (score 3), and 39% at a very high level (score 4). The *SM* levels were very similar among the groups at baseline, which shows a uniform colonization at the beginning of the study (Table 1).

Although there was no statistically significant difference among the treatments at any follow-up period regarding *SM* levels, overall reduction in *SM* counts was observed in all groups (Table 1; Figure 3). In general, the best performance occurred in the CHX group, followed by the F, XYL, and CHX+XYL groups (Table 1; Figure 3). *SM* recolonization was recorded in all groups 1 and 3 months after the first treatment appointment.

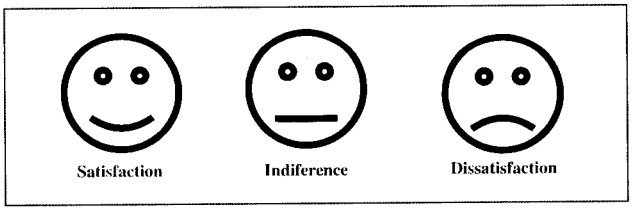

Figure 2. Faces scale.

Table 1. MEANS OF *STREPTOCOCCUS MUTANS* (*SM*) COUNTS AT FOLLOW-UP TIME POINTS AND MEAN VARIATION OF *STREPTOCOCCUS MUTANS* COUNTS BETWEEN FOLLOW-UP TIME POINTS AND BASELINE ACCORDING TO TREATMENT*

Treatment	SM counts													
	Baseline		15 ds			1 mo			3 mos			6 mos		
	N	Mean ±(SD)	N	Mean ±(SD)	Mean variation	N	Mean ±(SD)	Mean variation	N	Mean ±(SD)	Mean variation	N	Mean ±(SD)	Mean variation
CHX	17	3.00±0.86	14	2.07±1.87	0.93	16	2.19±0.96	0.81	14	2.36±0.85	0.64	11	2.27±1.12	0.73
CHX+XYL	14	3.10±0.95	12	2.67±0.80	0.47	12	2.67±0.68	0.47	6	3.17±0.52	-0.03	6	2.83±1.21	0.31
XYL	16	2.90±0.93	12	2.58±0.72	0.36	15	2.60±0.91	0.34	9	2.67±0.97	0.27	8	2.63±0.86	0.31
F	15	2.90±0.85	11	2.18±1.49	0.75	13	2.23±1.46	0.70	11	2.73±1.70	0.20	6	1.83±1.50	1.0
Total	62	-	49	-	-	55	-	-	40	-	-	31	-	-
					0.21			0.70			0.84			0.23

*$P>.05$ derived from nonparametric test: Kruskall-Wallis; N=number of children; mean=mean of SM score; mean variation=mean of variation compared to baseline; CHX=1% chlorhexidine varnish group; CHX+XYL=1% chlorhexidine varnish + 40% xylitol solution group; XYL=40% xylitol solution group; F=0.05% sodium fluoride group.

At the 6-month follow-up time point, reduction in *SM* counts was observed again in all groups, with the F group being the most effective (Table 1; Figure 3). Mean variations of *SM* scores, according to the follow-up periods, were higher between baseline and 15 days for all treatment groups. The CHX and F groups showed the highest variation (close to a 1-point score). It was not, however, statistically significant (Table 1). A decrease in *SM* levels at the follow-up time points can be seen in Table 2, but statistically significant differences were not found.

The plaque means were very similar among the groups at baseline, demonstrating a uniform ability for plaque formation within 24 hours among the groups (Table 3). All groups experienced reduction of plaque, although in small amounts and without statistically significant differences and regardless of the treatment, within 1 month. At 3 months, the XYL group returned to baseline scores and did not change at 6 months. The CHX group returned to baseline scores as well (Table 3; Figure 4).

After evaluating treatment acceptance, it was observed that, of the 10 CHX group children who answered the face scale, 9 were satisfied and 1 was dissatisfied. Of the 9 CHX+XYL group children, 6 were happy and 3 were indifferent. Of the 10 XYL group children, 9 were happy and 1 was indifferent; of the 9 F group children, 6 were happy and 3 were indifferent. The only child who did not like the treatment also exhibited poor behavior after the clinical examination. Overall, the results showed excellent children's acceptance of the tested agents.

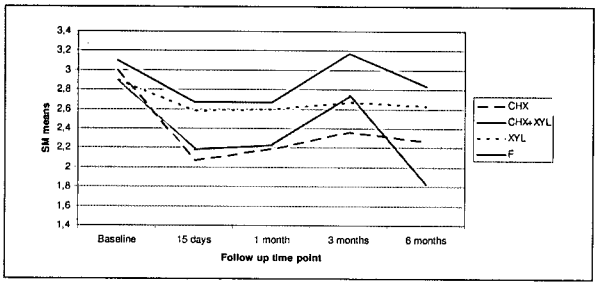

Figure 3. Means of *Streptococcus mutans* counts after the treatment (chlorehexidine [CHX]; CHX+xylitol [XYL]; XYL; and fluoride [F]), and during the follow-up time points (15 days, 1 month, 3 months, and 6 months).

Table 2. NO. AND % DISTRIBUTIONS OF CHILDREN ACCORDING TO *STREPTOCOCCUS MUTANS* LEVELS, TREATMENTS, AND FOLLOW-UP TIME POINTS*

Follow-up time point	Treatment	*Streptococcus mutans* levels										
		0		1		2		3		4		Total
		N	%	N	%	N	%	N	%	N	%	
Baseline	CHX	-	-	-	-	6	35	5	29	6	35	17
	CHX+XYL	-	-	-	-	5	36	2	14	7	50	14
	XYL	-	-	-	-	7	44	3	19	6	38	16
	F	-	-	-	-	6	40	4	27	5	33	15
15 ds	CHX	1	7	5	38	4	29	-	-	4	29	14
	CHX+XYL	-	-	2	17	4	33	2	17	4	33	12
	XYL	-	-	1	8	6	50	2	17	4	25	12
	F	2	18	2	18	2	18	2	18	3	27	11
1 mo	CHX	1	6	6	38	1	6	5	31	3	19	16
	CHX+XYL	1	8	-	-	5	42	2	17	4	33	12
	XYL	-	-	2	13	7	47	1	7	5	33	15
	F	2	15	2	15	3	23	3	23	3	23	13
3 mos	CHX	1	7	2	14	5	36	3	21	3	21	14
	CHX+XYL	-	-	-	-	2	33	1	17	3	50	6
	XYL	1	11	-	-	2	22	4	44	2	22	9
	F	1	9	1	9	3	27	1	9	5	45	11
6 mos	CHX	2	18	-	-	3	27	5	46	1	9	11
	CHX+XYL	-	-	-	-	3	50	1	17	2	33	6
	XYL	-	-	1	13	3	38	2	25	2	25	8
	F	1	17	2	33	1	17	1	17	1	17	6

* N=no. of children; %=percentage of children with the score in the group; CHX=1% chlorhexidine varnish group; CHX+XYL=1% chlorhexidine varnish + 40% xylitol solution group; XYL=40% xylitol solution group; F=0.05% sodium fluoride group.

The interviews verified that all the children had a cariogenic diet owing to high carbohydrate consumption, as well as high frequency. They all had prior contact with fluoride, although none had regular exposure to it. Evaluation of the parents' knowledge about the chemical agents used in this study showed that, of the 68 parents interviewed, only 3 were unaware about fluoride's role in tooth protection. Seven mothers had already heard about CHX. They did not know, however, that it could be applied on teeth. Only 2 mothers had already heard about XYL in chewing gum.

Only 1 XYL group child reported diarrhea on the first day of using XYL. No stains or irritated gingival tissue were observed on the teeth at any of the follow-ups in either the CHX group or CHX+XYL group.

Dropouts. Of the 68 selected children, 62 returned to treatment for the first follow-up (15 days), 55 returned for the second (1 month), 40 reported for the third (3 months), and 31 returned for the fourth (6 months). The highest dropout rate was observed for the CHX+XYL group (Table 2).

Discussion

Pretest discussion. According to Szklo and Nieto,[23] pretests have been developed to verify the feasibility and efficiency of the study procedures and, if necessary, correct the procedures before fieldwork begins. These authors also mentioned that this type of study is developed with a convenience sample under laboratory conditions. Our pretest was conducted exactly according to their guidelines. The development of a pilot study was rejected because it was not considered a necessary procedure once the pretest answered all the questions that emerged on the occasion of a study plan.

To train for the study, the clinical steps used in the study were reproduced in the pretest. For the same purpose, MSKB media (selective media for *SM*) was used in the pretest, although a standard *SM* strain was used to practice its manipulation and to observe the growth of these bacteria in a very selective growth medium.

The results showed no significant differences in colony counts across the plates under laboratory conditions. This suggests that, while the contact area of the tongue blade for inoculation could vary between 2.5 and 3.0 cm according to the child's age and mouth size, it should not affect the results.

Discussion. Strategies based on oral hygiene and noncariogenic diet are often insufficient to prevent the establishment of dental caries. Therefore, chemical caries control strategies are usually the main target of researchers.

Subjects between 2 and 5-years-old were the target of this research because:

1. The combination of CHX and XYL for children has not been previously studied.
2. Preventive measures have been considered to be the most economical and enduring alternatives to reduce dental caries.[24]

Although CHX is the most potent antimicrobial agent so far documented against *SM*, the observed suppression of these micro-organisms in the CHX group was not statistically significant in accordance of systematic review

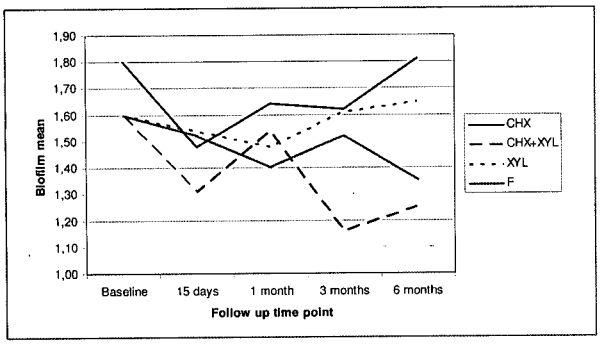

Figure 4. Means of plaque accumulation after the treatment (chlorehexidine [CHX]; CHX+xylitol [XYL]; XYL; and fluoride [F]) and during the follow-up time points (15 days, 1 month, 3 months, and 6 months).

information.[25] This result also corroborates previous studies in which 1% CHX varnish application was not very frequent nor was it done within a short time interval.[26-28] By contrast, frequent application of CHX within a relatively short time frame[29,30] in high concentrations[30,31] and the combination of frequent application and intensive professional mechanical tooth cleaning[32] resulted in statistically significant suppression. Therefore, it is likely that we would have seen significant reduction in *SM* if more CHX applications had been performed in the short time period of the study. It is important to emphasize that a predetermined protocol for CHX use does not exist. Recommended modes of administration of this agent in the literature have not been consistent.

SM recolonization has been reported after interruption of treatment with CHX, based on the frequency of therapy, vehicle used, and concentration of the agent.[26,33] Total *SM* recolonization was not observed at the follow-up time points even after low *SM* suppression and low frequency and concentration of CHX. The best reduction in plaque accumulation, although not statistically significant, seemed to occur in the CHX group. This finding agrees with another study, in which an initial decrease in plaque accumulation was observed followed by reduction that became less pronounced.[34]

In the XYL group, initial *SM* suppression was observed after 15 days when compared with baseline, and this suppression was sustained during all follow-ups (Table 1). These results, although not statistically significant, agree with other studies, in which *SM* reduction did not persist for a long time after XYL therapy,[14,35,36] probably because of the increase in selected XYL-resistant strains.[10]

The plaque index reduction previously reported[37] was not evident in the present study. This finding could be a consequence of low-frequency XYL use, even if used in high concentration. XYL has a dose-dependent effect.[38] Most of the studies evaluated this agent as applied with a high frequency (between 3 and 5 times a day) with chewing gum as the vehicle, which stays in the mouth longer when compared with other means of application. Another important point is that XYL's effectiveness, as well as that of sodium fluoride, was totally dependent on the mothers' compliance with the application regimes and could not be controlled by the dentist or hygienist.

Studies lacking combination therapy with CHX and XYL have been infrequently reported. The few available studies, however, reported better performance of these combined agents for *SM* suppression,[15,39] plaque index reduction,[15] and reduction of mother-child transmission of *SM*.[16] In this study, we did not observe the previously reported enhanced outcomes from this combination therapy. These weak results are probably a consequence of the same problems pointed out for the other treatment groups with these agents alone.

CHX varnish was chosen for the present study because the young children evaluated in this study did not have the capacity to spit out the mouthwash. Gel was also ruled out because of its bitter taste and the difficulty of maintaining the substance in contact with the teeth. A higher concentration of CHX varnish was also ruled out because of its bitter taste. This unpleased taste could represent a behavior problem for the young children used in the study. The authors tried to remove all possible factors which could interfere in the work with young children. Additionally, 1% CHX varnish presents good clinical outcomes in previous studies.[40] Foremost, the varnish requires fewer applications compared with mouthwash and gel. The XYL solution has not been used in clinical studies, and it was chosen because it would be very difficult to control gum chewing in young children. New vehicles are needed to effectively deliver xylitol at therapeutically effective levels over time[41].

The *SM* suppression observed in the F group illustrates the antimicrobial properties of fluoride, which had already been observed by Yoshihara et al.,[42] although the fluoride was tested

Table 3. MEANS OF PLAQUE ASSESSMENT AT FOLLOW-UP TIME POINTS AND MEAN VARIATION OF PLAQUE ASSESSMENT BETWEEN FOLLOW-UP TIME POINTS AND BASELINE ACCORDING TO TREATMENT*

Treatment	Plaque assessment												
	Baseline		15 ds			1 mo			3 mos			6 mos	
	N	Mean ±(SD)	N	Mean ±(SD)	Mean variation	N	Mean ±(SD)	Mean variation	N	Mean ±(SD)	Mean variation	N	Mean ±(SD)	Mean variation
CHX	17	1.80±0.29	14	1.48±0.33	0.26	16	1.64±0.34	0.12	14	1.62±0.45	0.10	11	1.81±0.25	-0.06
CHX+XYL	14	1.60±0.37	12	1.31±0.22	0.22	12	1.54±0.23	0.04	6	1.16±0.49	0.33	6	1.25±0.38	0.25
XYL	16	1.60±0.30	12	1.54±0.02	0.02	15	1.48±0.43	0.13	9	1.61±0.41	0.00	8	1.65±0.37	-0.09
F	15	1.60±0.37	11	1.52±0.06	0.06	13	1.40±0.32	0.17	11	1.52±0.45	0.09	6	1.35±0.22	0.10
Total	62	-	49	-	-	55	-	-	40	-	-	31	-	-
					0.39			0.87			0.70			0.17

*$P>.05$ derived from nonparametric test: Kruskall-Wallis; N=no. of children; mean=mean of plaque scores; mean variation=mean of variation compared to baseline; CHX=1% chlorhexidine varnish group; CHX+X=1% chlorhexidine varnish + 40% xylitol solution group; XYL=40% xylitol solution group; F=0.05% sodium fluoride group.

in higher concentrations. The observed *SM* suppression between 3 and 6 months, even though not statistically significant, corroborates the idea that the agent's performance is highly dependent on its use. At the 3-month follow-up, the bottle containing the treatment solution was replaced by a new one. This fact could have worked as a motivating factor for the parents to continue applying fluoride solution throughout the entire study period.

Plaque assessment was based only on the surface area covered with plaque. The quality of plaque in these areas was not evaluated. Moreover, it is known that *SM* levels and plaque accumulation have an important relationship with carbohydrate consumption.[43] In this study, it was observed that all children had a cariogenic diet, which could interfere with the agent's performance. Even though dietary instructions were given at the beginning of the study, changing one's habits is a difficult task, especially with only one motivation session.

A nonparametric statistical analysis was performed for microbiological and clinical data without use of means of scores. The means of scores were used only in tables and graphics to illustrate the results.

Initially, the sample size of this study was determined to be 100 children, 25 in each group. After subject selection, however, only 68 children (~47%) out of 144 were included in the study based on their high *SM* levels. It is difficult to compare our *SM* colonization prevalence with other studies mainly because of the different methodologies applied, especially the different selective *SM* media used.

The spatula method was chosen because it was considered one of the simplest of laboratory tests, requiring fewer steps than other lab tests. In this method, the saliva sample does not need to be diluted before inoculating the media. This characteristic makes the test most appropriate for epidemiological studies or dental practice. Another important advantage is that a spatula or tongue blade is used to collect the saliva sample, which makes it practical for small children, compared with other classical methods in which the saliva has to be collected after stimulation.

The MSKB agar medium, more selective for recovery of *SM* than the mitis salivarius bacitracin (**MSB**) agar medium, was used in this study. MSB is not exclusively selective for *SM*, and the colonial morphology is not a foolproof method of distinguishing *SM* from the other streptococcal species. The authors showed that recovery of *SM* on MSKB was 13% less than on MSB.[20] This fact could have determined that children with medium levels of *SM* would have been classified as low-level, and thus could have been excluded from the study. MSKB medium, however, was chosen for its long shelf life, high capacity to select *SM* colonies, and low number of non-mutans colonies that could be visually confused with *SM*—important characteristics for more precise colony counting.[20]

The Faces Scale was used to evaluate pain through a self-report. This scale was chosen because it is an attractive approach, simple, and quick to conduct.[22] The excellent acceptance of CHX by children, regardless of its bitter taste, is probably due to the varnish vehicle, which allows low concentration and safe application without contact with the tongue.[12] This result would probably not have been achieved had the gel vehicle been used. The other groups' acceptance was probably due to either the sweet taste of the XYL or the absence of the taste of sodium fluoride, in addition to the fact that the children felt more comfortable with their mothers' applying the agents. The good acceptance of chlorhexidine varnish by children had already been reported.[44]

Future studies should be performed to test the effects of more frequent applications of CHX and XYL.

Conclusions

Based on this study's results, the following conclusions can be made:

1. Overall reduction in *Streptococcus mutans* counts was observed in all treatment groups with the 1% chlorhexidine varnish group presenting the most reduction.
2. All treatment groups presented reduction of plaque accumulation with the 0.05% sodium fluoride group showing the most plaque reduction.
3. Effective antimicrobials and delivery vehicle systems are needed to reduce plaque and cariogenic bacteria in young children.

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