

The Effects of Chlorhexidine Gel on *Streptococcus mutans* Infection in 10-month-old Infants: A Longitudinal, Placebo-controlled, Double-blind Trial

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Abstract

Purpose: This study investigated the long-term effects of 0.2% chlorhexidine gel, used as a weekly brush-on gel, on *Streptococcus mutans* infection in 10-month-old infants.

Methods: The investigation followed the criteria of a placebo-controlled, double-blind, longitudinal clinical trial. Infants were recruited at birth and oral microbiological swabs were taken at 3 monthly intervals, together with medical, dental, dietary and brushing histories. Children who were found to be colonized with *S mutans* were randomly assigned to either the chlorhexidine-gel group (N=50) or placebo gel group (N=46), and parents were instructed to brush the gel on the teeth once per week for 12 weeks. In another control group (N=210), infants did not use either chlorhexidine or placebo gels. Saliva samples were cultured using *S mutans*-selective tryptone-yeast extract-cysteine-sucrose-bacitracin (TYCSB) agar. The mean age of the children was 10.2±2.6 months at the start of the trial and subjects were followed until the ages of 18 months.

Results: In the children with initial low *S mutans* counts of <300 CFU/mL, there was a significant percentage reduction in *S mutans* counts in the chlorhexidine-gel group compared to the placebo gel and no-gel control groups after 3 months of weekly gel brushing. However, no significant differences with the placebo group were observed after 15 months of follow-up. There were 39 children (41%) who achieved reduction of their *S mutans* to 0 CFU/mL. Compared to those who remained infected with *S mutans*, these children had higher toothbrushing frequencies ($P<.001$) and toothpaste use ($P<.001$), as well as lower frequencies of daily feeds ($P<.01$), and lesser weekly frequencies of sweet solids and liquids ($P<.001$).

Conclusions: Children with relatively low initial *S mutans* counts (<300 CFU/mL) showed a reduction in *S mutans* counts in the first 3 months when 0.2% chlorhexidine gel was brushed on the teeth weekly. No differences were observed when compared with the placebo and no-treatment groups at later follow-up periods. (*Pediatr Dent.* 2003;25:215-222)

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The bisbiguanide chlorhexidine gluconate is a highly effective agent against the cariogenic bacteria *Streptococcus mutans* (*S mutans*).¹ At concentrations ranging from 0.1% to 40% in solutions, gels, chewing tablets, and varnishes,¹⁻³ chlorhexidine gluconate has been shown to reduce *S mutans* in children and adults who have high caries risks.⁴⁻¹⁰ Continuous and regular long-term use

of chlorhexidine gel has not been associated with major shifts in the microbial profiles of dental plaque or with minimal changes in susceptibilities.¹¹⁻¹² Side effects of long-term applications are minimal and limited to development of removable yellowish-brown stains and, occasionally, complaints of objectionable taste.¹³

It is now established that the time of colonization of cariogenic bacteria *S mutans* in an infant's mouth is of clinical significance in that the younger the infant is at the time of colonization, the greater is his or her caries risk.¹⁴⁻¹⁷ Although early investigators reported that there is a "window of infectivity" at around a mean age of 27 months,¹⁸ the authors found in previous studies that over 30% of children as young as 3 months of age were already infected with the cariogenic bacteria.^{19,20} The authors hypothesize that reduction of *S mutans* infection in infants is possible using weekly brush-on application of chlorhexidine gel on the teeth. Removal of the cariogenic bacteria in young children is theoretically advantageous in that early clearance of *S mutans* in mouths which are undergoing initial stages of microbial development may have a greater potential for long-term elimination of the bacteria.

The purpose of this study, therefore, was to determine the long-term anti-*S mutans* effect of a weekly brush-on application of 0.2% chlorhexidine gel in young infants.

Methods

Subjects

Ethical approval for the present study was obtained from relevant human ethics committees of the University of Queensland and the Mater Mothers' Hospital, both in Brisbane, Australia. Infants were recruited randomly at birth from the Mater Mothers' Hospital. The majority of the recruited infants were Caucasians. These infants were recalled for dental examinations at 3 monthly intervals, up to the age of approximately 18 months. The consent rate for this study was over 90%.

The interviews and examinations were conducted by one of the authors (AKLW). At each examination, an interview based on validated questionnaires²¹ was conducted with mothers to obtain and update information concerning medical history, feeding, diet, and oral hygiene. The number of erupted teeth and plaque levels were checked during examinations. Plaque levels were assessed qualitatively as visible vs nonvisible, as well as quantitatively, to determine whether <1/3, 1/3, and >1/3 of dentition showed the presence of plaque.^{22,23}

All subjects enrolled in the study were given routine oral hygiene using a child's toothbrush, employing the circular-scrub method. A new child's toothbrush (My First Colgate Extra-soft, Colgate, Australia) was given to every parent at the start of the study.

At every follow-up visit, microbiological samples were obtained by swabbing infants' teeth, oral mucosa, and alveolar ridges with sterile cotton tips. Similar swabs were obtained from the mothers. Each saturated tip was placed into a sterile vial containing phosphate-buffered saline (PBS) at pH 7.2 and transported to the laboratory at 4°C. Each saturated swab held 0.1 mL saliva. Samples were sonicated for 30 seconds and aliquots of 50 mL diluted samples were spread evenly onto *S mutans*-selective tryptone-yeast-

cysteine-sucrose-bacitracin agar (TYSCB, BioMerieux, Sydney, Australia).^{19,20,24} Samples were plated in triplicate, incubated at 37°C under atmospheric conditions (10% H₂, 10% CO₂, and 80% N₂) using commercial AnaeroPacks (Mitsubishi Gas Chemical Inc, New York) for 72 hours. The colonies were counted using a colony counter. The number of *S mutans* was calculated as colony-forming units per mL of saliva (CFU/mL).

Bacterial identification was performed by means of colony morphology, Gram stains and biochemical tests using Rapid Strep ID32^{25,26} (API, BioMerieux Vitek, Marcy-l'Etoile, France). Control plates of the laboratory strain *S mutans* (NCTC 10449) were incubated along with sample plates to confirm the identification and growth of *S mutans* on TYSCB agar. The biochemical analyses for speciation using Rapid Strep ID32 were performed for every batch of microbiological analysis. For each analysis, random colonies morphologically identified as *S mutans* were selected from at least 6 sample plates. The Rapid Strep ID identifies all the common strains of *S mutans*.^{25,26}

Chlorhexidine and placebo gels

A commercial preparation of 0.2% chlorhexidine gluconate-gel (Periogard Gel, Colgate, Australia) was used for the study. The placebo gel, which was prepared by Colgate Laboratories (Labrador, Queensland, Australia), was identical in color, consistency, and composition to the chlorhexidine gel, except that it did not contain chlorhexidine gluconate. The gels were dispensed in identical 30 mL plastic bottles, which were each labeled with a number code. The code was broken at the end of the study by an independent person not involved in the study.

The chlorhexidine and placebo gels were first tested to validate their respective effects on *S mutans* using a standard test.²⁷ Briefly, *S mutans* (NTCC 10449) was cultivated on trypticase soy yeast extract agar (Oxoid, NSW, Australia) supplemented with 5% sheep blood. A light suspension was prepared in sterile saline and swabbed over the surface of the agar plates. A blank antibiotic disc (Oxoid, Basingstoke, England) was used to take up either the 0.2% chlorhexidine gel or the placebo gel. The discs were applied to the surface of the agar plates and incubated for 48 hours in a candle jar at 37°C.

Validation of anti-*S mutans* activity of chlorhexidine gel

The standard tests for inhibitory activity demonstrated clear zones of inhibition of the *S mutans* around the discs impregnated with the chlorhexidine-gel. In contrast, there were no zones of inhibition in the placebo gel, indicating that it had no inhibitory effects on *S mutans*.

Subject groups

All dentate infants who showed *S mutans* in 2 consecutive samples were considered to be infected with the bacteria and were invited to participate in the trial. In addition, a group of infants whose mothers did not consent to use the

Table 1. Demography and Gel Usage

Variable	No gel control (N=210)	CHX (N=50)	PBO (N=46)	P value*
Gender (%)				
Females	41%	40%	39%	NS†
Males	60%	60%	61%	
Group (%)				
Preterm	33%	32%	15%	NS†
Full-term	67%	68%	85%	
Gestation age				
Mean (wks)	37.2±4.5	37.2 ±4.1	38.9 ±2.3	NS‡
Birthweight				
Mean (kg)	2.9±1.1	2.9 ±1.0	3.4 ±0.8	.036‡
Parity§				
Mean	1.64±1.21	1.72±1.34	1.72±1.07	NS ‡
Initial <i>S mutans</i> (CFU/mL)				
Mean	261.7±146.8	851.1±451.8	356.5±929.9	NS‡
No. of erupted teeth				
Mean	6.3±3.7	4.21 ±3.53	5.24 ±3.76	NS‡

*Comparing chlorhexidine gel vs placebo gel users; NS=not statistically significant.

†Chi-square test.

‡Mann Whitney test/Kruskal Wallis test.

§Parity is defined as "the total number of pregnancies; the classification of a woman by the number of live-born children and stillbirths she has delivered at more than 28 weeks of gestation."³³

gel, but who consented to be followed-up for microbiological examination, served as a no-gel control group. Signed informed consent was obtained from all the mothers in the study.

The study was double-blinded, in that both the investigators and mothers were unaware as to whether the gel given was chlorhexidine or placebo. Children were assigned to placebo or chlorhexidine gel groups by randomly drawing a bottle from a bag containing equal numbers of placebo and chlorhexidine gel bottles which were individually coded with numbers. The number codes were broken at the end of the study period. All mothers were given instructions to apply 3 drops of gel on a child's toothbrush and to brush all erupted teeth with the gel once per week for 12 weeks. They were advised not to rinse the children's mouths or brush the teeth again within an hour of gel application.

Mothers of the children in the no-treatment control group who were not given gels were instructed in routine oral hygiene instructions. They were advised to use a child's toothbrush and a small pea-size amount of low-dose (400 ppm) fluoride junior toothpaste (My First Colgate Fluoriguard, Colgate, Australia) to brush their children's teeth daily, using the gentle circular-scrub method.

Table 2. Direct Statistical Comparison of the Difference in *S mutans* Levels Between the Chlorhexidine Gel-treated Group and Placebo Gel-treated Group

	Mean level±SD of <i>S mutans</i> (CFU/mL)		P value*
	Chlorhexidine (N=50)	Placebo (N=46)	
Pregel treatment	176±158	183±180	NS
Immediately postgel treatment	116±121	95±83	NS
3 mos postgel treatment	33±35	50±109	NS
6 mos postgel treatment	161±245	110±146	NS
9 mos postgel treatment	172±152	181±214	NS
12 mos postgel treatment	135±209	1,914±3,133	.037
15 mos postgel treatment	350±158	370±439	NS

*Mann-Whitney test; P>.05=NS (not statistically significant)

Statistical analysis

SPSS version 10.0.5 was used for statistical analyses. Non-parametric analyses were performed using chi-square, Mann Whitney, Kruskal Wallis tests, Spearman's rank-order correlation, and logistic regression.

Results

Demography

Table 1 shows the demography of subjects enrolled in the study. A total of 96 (23 preterm, 73 full-term) infants participated in this trial. Fifty subjects (30 males, 20 females) were given chlorhexidine gel, 46 (28 males, 18 females) were given the placebo gel, and 210 infants (85 females, 125 males) did not receive either the chlorhexidine or placebo gel. As shown in Table 1, there were no differences in gestational ages or female:male ratios among the 3 groups of subjects in the study. The distribution of ethnicity and socioeconomic groups were also similar among the groups.

Chlorhexidine gel and placebo gel usage

Only children with erupted teeth were recruited into the study. The mean starting age of gel application was 10.2±3.6 months for chlorhexidine and 10.2±2.4 months for placebo (P>.1; Table 1). The numbers of reported missed weekly applications were small and similar in both chlorhexidine and placebo gel groups (2.2±2.4 times vs 3.0±2.9 times, respectively; P>.05). The chlorhexidine and placebo gels were well accepted by the infants. Side effects were reported by 4 mothers whose infants were given the placebo gel, and none were reported by those on chlorhexidine gel (P<.04). Of those with side effects, 2 reported a yellowish stain on the infants' teeth, 1 reported extreme irritability, and one reported a localized erythema on gingival margins.

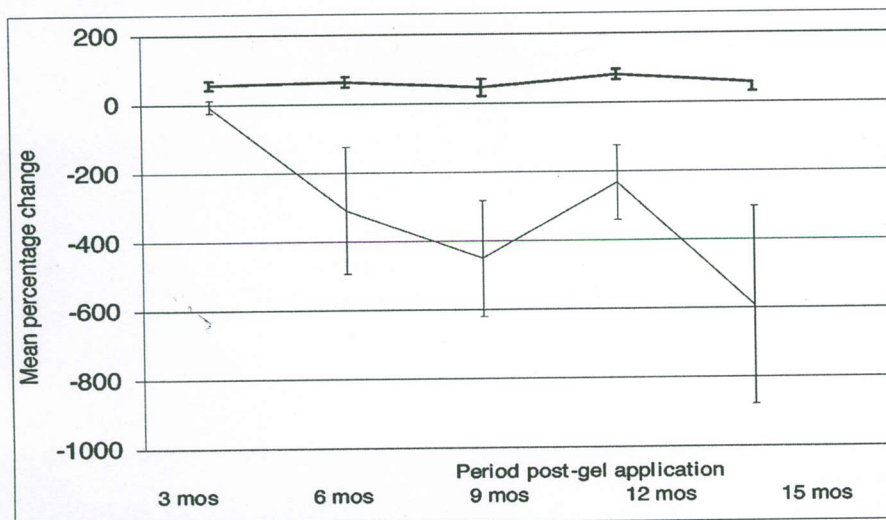


Figure 1. Mean percentage changes of *S mutans* from baseline values in chlorhexidine gel subjects with different initial levels of *S mutans* (— <300 CFU/mL; - - > 300 CFU/mL; vertical bars represent \pm SE).

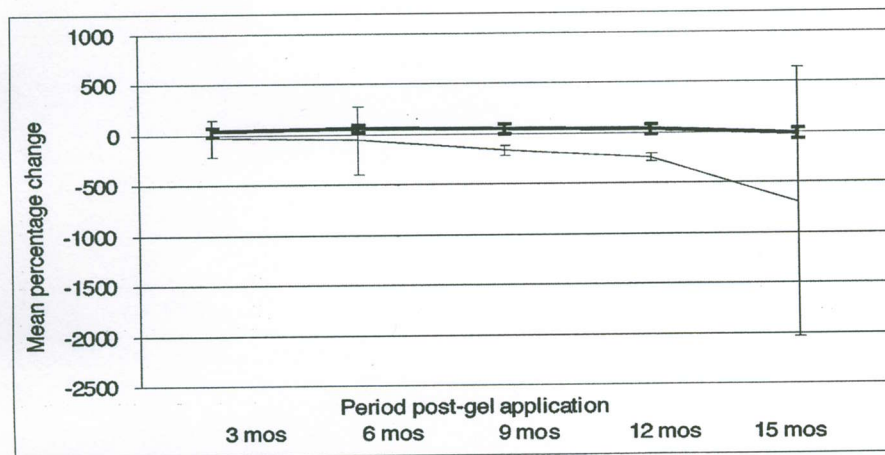


Figure 2. Mean percentage changes of *S mutans* from baseline values in placebo gel subjects with different initial levels of *S mutans* (— <300 CFU/mL; - - > 300 CFU/mL; vertical bars represent \pm SE).

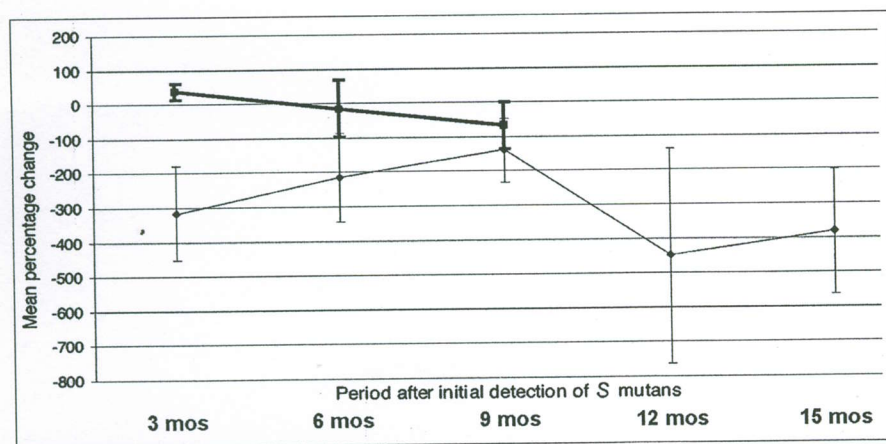


Figure 3. Mean percentage changes of *S mutans* from baseline values in subjects who did not receive placebo or chlorhexidine gels (— <300 CFU/mL; - - > 300 CFU/mL; vertical bars represent \pm SE).

Direct comparison of *S mutans* levels between chlorhexidine gel and placebo gel treatment groups

As shown in Table 2, a direct comparison of *S mutans* levels between chlorhexidine gel and placebo gel groups before gel treatment, immediately following gel treatment, and 3-, 6-, 9-, 12-, and 15-months following gel treatment did not reveal any significant differences, except at 12 months following treatment. The mean *S mutans* level of the placebo gel group at 12 months following gel treatment was significantly higher than the chlorhexidine-gel group ($1,914 \pm 3,133$ vs 135 ± 209 , $P < .04$).

Changes in *S mutans* levels in the infant groups

Infants were stratified according to their initial *S mutans* levels as <300 CFU/mL or >300 CFU/mL.

Figure 1 shows the percentage changes of *S mutans* in children who had been treated with the chlorhexidine gel. As shown in Figure 1, there was a significant reduction in the percentage of *S mutans* after 3 months of chlorhexidine gel use ($P < .025$) for infants with initial *S mutans* levels of <300 CFU/mL. This reduction in *S mutans* was sustained for up to 15 months after the stopping of chlorhexidine gel use, although the percentage reduction was not significant after 3 months from cessation. In the case of children with *S mutans* of >300 CFU/mL, there was no significant change in bacterial numbers throughout the period of study when chlorhexidine gel was used.

Figure 2 shows the percentage changes of *S mutans* in children who have been treated with the placebo gel. As shown in Figure 2, reductions in percentages of *S mutans* were not statistically significant among infants who were given the placebo gel, regardless of the initial levels of *S mutans* (Figure 2).

Figure 3 shows the percentage changes of *S mutans* in children who have not received treatment with either chlorhexidine or placebo gel. In this group, there were not significant percentage changes in *S mutans* levels throughout the period of study (Figure 3).

Habits of infants who achieved reduction of *S mutans* to 0 CFU/mL compared to those who remained infected

As shown in Table 3, altogether there were 39 children (41%) who achieved reduction of their *S mutans* counts to a level undetectable by TYCSB agar. These infants were assumed to have 0 CFU/mL of *S mutans*. Of these, 22 (56%) brushed with the chlorhexidine gel and 17 (44%) brushed with the placebo gel ($P>.1$). As shown in Table 3, although there were no differences in the maternal salivary levels of *S mutans* between the group who achieved reduction of *S mutans* to 0 CFU/mL compared to the group who remain infected, there were significant differences in the oral habits between the groups. Greater percentages of children who achieved reduction of *S mutans* to 0 CFU/mL showed nonvisible levels of dental plaque when they presented for their recall examinations ($P<.007$), and have higher frequencies of tooth-brushing ($P<.001$) and toothpaste use ($P<.001$), compared to those who remained infected with *S mutans*. Furthermore, the children who did not achieve reduction of *S mutans* to 0 CFU/mL had higher frequencies of daily feeds ($P<.01$), being put to bed with a bottle of milk ($P<.004$), and weekly sweet solids and liquids ($P<.001$), and have reported a greater prevalence of sharing food, drinks, and utensils with others ($P<.001$) and dummy sucking ($P<.04$; Table 3).

Discussion

Chlorhexidine is one of the most potent chemotherapeutic agents currently available against the mutans streptococci group.^{1,28} Treatment with chlorhexidine gel for short time periods has been shown in human studies to reduce *S mutans* to low levels in saliva and dental

Table 3. Habits of Infants Who Achieved Reduction of *S mutans* to 0 CFU/mL Compared to Those Who Remained Infected

	<i>S mutans</i> 0 CFU/mL (N=39)	<i>S mutans</i> >0 CFU/mL (N=57)	<i>P</i> value*	OR (95%CI)
Maternal salivary level of <i>S mutans</i> (CFU/mL initial±SD)	5.5×10 ⁴ ±5.1×10 ⁴	5.6×10 ⁴ ±6.5×10 ⁴	NS†	
Placebo group	44%	51%	NS*	-
Chlorhexidine group	56%	49%		
Plaque present				
Before gel use	59%	70%	NS*	-
After gel use	33%	61%	.007*	1.8 (1.1-3.0)
Oral hygiene habits				
Tooth-brushing (times per day)				
0	3%	12%	.001*	-
1	33%	58%		
2	62%	21%		
3	3%	0%		
Consistent toothpaste use	82%	42%	<.001*	6.3 (2.4-16.6)
Fluoride supplement	51%	0%	NS*	
No. of teeth present				
Mean±SD	4.5±3.5	5.0±3.5	NS†	
Feeding habits				
Weaned	10%	3%	NS*	-
Breastmilk	28%	44%		
Formula	54%	39%		
Both breastmilk and formula	8%	14%		
No. of feeds (per day)				
Mean±SD	2.5±1.5	3.5±2	.014†	-
Put to sleep with milk				
No	72%	35%	.004*	-
Yes	28%	65%		
Sweet solids per week (mean±SD)	2.0±2.2	5.7±4.84	<.001†	
Sweet fluids per week (mean±SD)	3.4±2.7	5.3	<.001†	
Shared food/drinks with others (%)	31%	81%	<.001*	2.6 (1.6-4.2)
Shared utensils (%)	31%	79%	<.001*	3.6 (1.6-4.2)
Dummy sucking (%)	28%	49%	.04*	1.7 (1.0-3.1)

*Chi-square test; NS=not statistically significant.

†Mann Whitney test.

plaque.^{1,2,29-31} Such studies have been extended to examine the effects of chlorhexidine on dental caries, and these reported a caries rate reduction of around 50% in children with moderate levels of *S mutans* to more than 80% in children with high levels of the bacteria.^{32,33}

However, to date, there have been very few previous dental studies on the antimicrobial effects of chlorhexidine which have enrolled the use of a placebo control, and no clinical investigations are available which have followed strict criteria of a double-blind, placebo-controlled trial. Furthermore, previous clinical trials were performed mainly in older children and adults, and only 3 small studies have employed children under the age of 5 years.^{1,7,34} By contrast, the present study met all requirements of a placebo-controlled, double-blind, controlled trial. In addition, the longitudinal nature allowed a relatively long-term evaluation of the effects of chlorhexidine, which was not performed in the majority of previous studies. The present study is further unique in that it explores the possibility of elimination of *S mutans* by chlorhexidine in the early stages of its colonization and plaque development. The authors hypothesize that this has important clinical implication in that timely suppression of *S mutans* in the early stages of oral microbial establishment may result in its long-term elimination from the oral cavity.

The results of the present study show that, in infants with relatively low initial *S mutans* counts (<300 CFU/mL), after 3 months of weekly brushing with the 0.2% chlorhexidine gel, the *S mutans* percentage reductions were significantly reduced compared to the placebo and no-gel groups. In some infants, the suppression was sustained up to the age of 18 months but the results were not statistically significant.

The reasons for the inability of once weekly toothbrushing with 0.2% chlorhexidine gel for 3 months to suppress colonization of *S mutans* in the long-term may be related to the preparation, dosage, frequency of application, method of application used, and compliance. In the present study, the authors used a gel preparation which was found to be highly acceptable among the majority of children. Compliance was found to be high, and only a few parents reported missed applications, which were, at the most, for only 2 weeks. Therefore, the authors speculate that, in the present study, the lack of a long-term effect of chlorhexidine may be due to the relatively long period of time between applications in relation to the low concentration. Thus, it is possible that the recolonization of the teeth by *S mutans* occurs under these circumstances, particularly when oral hygiene is poor and the diet is high in sugar. This hypothesis is supported by numerous previous studies which reported that recolonization of *S mutans* occurs after cessation of chlorhexidine treatment.^{12,30,36,37} Other possibilities, such as adaptation of the bacteria to chlorhexidine, are unlikely for the relatively short duration of chlorhexidine use. Based on the present results, for long-term effects, it may be necessary to increase the frequency of application of chlorhexidine or increase its concentration. Alternatively, the duration of application may need

to be long enough to cover the duration of potential infectivity by *S mutans*.

Of great interest is the fact that decreases in *S mutans* counts were also noted in the placebo group, which suggested the presence of other influencing factors besides chlorhexidine, which can reduce *S mutans* in the study subjects. To determine these factors, the authors compared the brushing and feeding habits of children who achieved elimination of *S mutans* (0 CFU/mL), as indicated by undetectable levels of the bacteria in the microbiological tests, and the children who continued to harbor *S mutans*. The analyses revealed that children who achieved reduction of *S mutans* to 0 CFU/mL were those who had better oral hygiene and less dental plaque. Nearly all mothers of these children reported brushing the children's teeth with toothpaste at least once per day and, in more than 60%, twice per day. This study's results thus support the authors' recent investigations, which demonstrated that there is less *S mutans* in children who have good oral hygiene.^{19,20} The findings are further supported by the study of Axelsson et al, in young adolescents which showed that *S mutans* levels can be reduced by tooth-brushing alone.³⁵

In addition, the infants who showed reduction of their *S mutans* counts to 0 CFU/mL also had better feeding habits compared to those who continued to harbor the bacteria. They had fewer feeds per day ($P < .05$), were less likely to be put to bed with a bottle of milk ($P < .01$), and had less than half the number of total daily sugar exposures ($P < .001$). Thus, it is most likely that a combination of good dietary and oral hygiene habits have reduced the *S mutans* counts to undetectable levels in these children.

Previous studies have also suggested that recolonization of the teeth with *S mutans* may occur after chlorhexidine treatment, particularly in those with high initial bacterial counts.^{12,30,36,37} In this regard, in addition to poor oral hygiene and dietary habits, one of the reasons why some children continue to remain infected with *S mutans* may be reinfection from another individual who is likely to be the mother, particularly if she has high *S mutans* counts herself and poor oral health status.^{19,20} On the other hand, in the present study, there were no differences in maternal salivary levels of *S mutans* for the children who achieved a reduction of *S mutans* to 0 CFU/mL compared to those who continued to harbor the bacteria.

Conclusions

1. Weekly brushing with 0.2% chlorhexidine gel for 3 months in 10-month-old infants reduces *S mutans* infection in children with initial counts of <300 CFU/mL for up to 15 months, but statistically significant results were obtained only after the first 3 months compared to the placebo gel.
2. Regardless of the treatment received, the infants who reduced their *S mutans* counts to 0 had their teeth brushed at least once per day, ate less snacks, and had significantly less daily sugar exposure compared to those children who remained infected.

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