

# A longitudinal study of 0.2% chlorhexidine gel for removal of *mutans streptococci* infection in preschool children

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## Abstract

**Background:** The purpose of the present longitudinal study was to determine the effectiveness of 0.2% chlorhexidine (CHX) in removing *mutans streptococci* (MS) in a treatment cohort of caries-free, preschool children.

**Methods:** Thirty-six children with mean age 38.6±15.9 months who had MS infection brushed once daily with 0.2% chlorhexidine (CHX) gel for a period of 6–12 months. The children were examined every three months to assess their oral hygiene and dietary habits and MS status using a commercial microbiological kit.

**Results:** The percentages of children who eliminated MS from their mouths increased from 28 per cent after three months to 48 per cent after six months and over 70 per cent after 12 months of gel use ( $p=0.039$ ). Compliance of brushing with CHX gel was significantly associated with MS removal after six months gel use ( $p<0.02$ ). At the three and six month recalls, sugar snacks and increased sugar frequency were associated with continued presence of MS ( $p=0.03$  and  $p=0.007$ ). After three months cessation of CHX use, 13 (45 per cent) remained MS free ( $p=0.003$ ).

**Conclusions:** This pilot study involving caries-free preschoolers with MS shows the potential of chlorhexidine gel to remove MS for the limited duration of study.

**Key words:** Chlorhexidine, *mutans streptococci*, early childhood caries, preschoolers.

**Abbreviations and acronyms:** CHX = chlorhexidine; ECC = early childhood caries; MS = *mutans streptococci*.

(Accepted for publication 7 July 2006.)

caries, the aetiology of ECC is multifactorial, and is centred on the oral colonization of cariogenic bacteria (*mutans streptococci*, MS) which ferments dietary sugars to produce acids that demineralize the tooth surface.<sup>1</sup>

The high rate of MS infection of children found in our previous longitudinal studies may help explain why it is relatively difficult to control ECC.<sup>2,3</sup> Their early presence in a child's mouth before tooth eruption suggests that as soon as other cariogenic factors (such as dietary sugars) become available, onset of caries is likely to be rapid. Thus, timely removal of MS is likely to reduce caries risk. In this regard, although a few antibacterial agents are promising, chlorhexidine gluconate (CHX) has been shown to be particularly efficacious against the cariogenic bacteria.<sup>4</sup>

As an anti-caries agent, CHX is used at concentrations ranging from 0.1 to 40 per cent in solutions, gels, chewing tablets and varnishes.<sup>5-17</sup> It is a safe antiseptic with few side effects, and its long-term use is associated with only minimal shifts in the oral microbial plaque profiles and only slight changes in susceptibilities.<sup>18,19</sup> The majority of previous clinical trials which were performed mainly on older children, teenagers and adults, and employed mainly CHX varnish as the vehicle, demonstrated anti-caries effects ranging from 11 to 68 per cent.<sup>6-8</sup> A meta-analysis of selected studies gave an overall caries inhibiting effect of CHX of around 46 per cent.<sup>8</sup>

To date, only two studies have explored the use of CHX to remove MS in preschool children, and another two on the anti-caries effect of CHX.<sup>9-12</sup> Twetman and Grindejord investigated the reduction of MS in the mouths of toddlers for one month after a two-week duration of twice daily application of 1% CHX gel.<sup>9</sup> The authors reported that after three months, MS reduction was not significant when compared with the baseline level. Wan and co-workers also reported that there was removal of MS from the mouths of toddlers after brushing for three months using a once-weekly 0.2% CHX gel.<sup>10</sup> However, this regimen of brushing was effective mainly for those children with relatively low MS counts of approximately  $10^3$  per ml of saliva.<sup>10</sup> The effect of CHX gel flossing on proximal caries

## INTRODUCTION

Early childhood caries (ECC), which affects predominantly socio-economically disadvantaged children, is one of the most significant conditions in paediatric dentistry today.<sup>1</sup> As in other forms of dental

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reduction in preschoolers was examined by Gisselsson *et al.*, who reported that after nine years post-treatment, an anti-carries effect was still observed in the CHX-treated group.<sup>11</sup> Similarly, Pienihakkinen and Jokela found that high risk two year old children who were given CHX varnish treatment applied four times a year, combined with other preventive methods, showed reduction in caries for up to three years post-treatment.<sup>12</sup> On the other hand, Plotzitz *et al.* recently reported that CHX varnish applied to 23 high-risk children did not alter their MS status compared to a control group who did not receive the varnish.<sup>13</sup>

The aim of the present study, therefore, was to determine the long-term effectiveness of CHX in the removal of MS in a cohort of preschool children who were infected with MS but do not have dental caries.

## SUBJECTS AND METHODS

The study was approved by The University of Queensland Human Research Ethics Committee. The cohort of children was recruited from birth as part of a longitudinal study of *mutans streptococci* (MS) colonization and the children were already enrolled in three month review with brushing and dietary advice.<sup>2,3</sup> In line with ethical requirements, children who were found to have acquired MS were offered CHX as treatment to remove the cariogenic bacteria. The present investigation thus reports on the sub-group of children who received CHX treatment. The study was not designed as a placebo-controlled trial, and effectiveness of the gel was determined by examining compliance of gel use in relation to MS removal.

A single examiner (VL) performed the dental follow-up visits at the School of Dentistry Paediatric Dentistry clinics. Social, family, medical and dental histories were taken to re-check previous record and update current conditions. At every three month recall, the frequency of daily toothbrushing and a three-day dietary history was given to each parent to determine the mean number of sugar-containing items consumed daily. The soft tissues and teeth were examined using mirror and dental explorer, and the results recorded in standardized charts.

Intra-examiner consistency for recording of dental caries and enamel hypoplasia was performed using duplicate examinations of 20 children of similar ages from intra-oral photographic records.<sup>14</sup> Examiner consistency, using the kappa statistic was found to be greater than 0.85.<sup>14</sup>

All children in the study were treated with 0.2% CHX gel (Periogard®, Colgate, Australia), using a regimen of once-daily brushing with two drops of the gel, after routine daily toothbrushing with child toothpaste (My First Toothpaste®, Colgate, Australia).

Routine advice regarding diet and dental health was also provided to all parents. The compliance of gel use was recorded as “good” if the parents reported one instance or less of non-use of the gel per week, “moderate” if the parents missed two or three times per

week, and “poor” if they missed more than three times per week.

## Test for MS infection

A commercially available microbiological test in which bacterial growth is semi-quantitated on selective agar for MS (CRT Strip mutans test®, Ivoclar, Melbourne, Australia) was used to determine the presence or absence of MS infection.<sup>14,15</sup> In this test, a plaque sample was obtained by swabbing all tooth surfaces using a sterile, cotton-tipped swab which was then spread across the surface of the agar provided in the kit. The jars were screwed tightly after addition of a pellet of sodium bicarbonate to increase the carbon dioxide content, and the kits were incubated at 37°C. After 48 hours, the kits were removed from the incubator, and the amount of bacterial growth assessed using reference charts provided by the manufacturer. Children were considered MS positive only when two consecutive three-monthly tests showed positive results. Similarly, children were considered MS negative only when two consecutive three-monthly tests showed negative results, e.g. for a child to be considered negative at three months, a second negative test at six months must also be demonstrated.

The socio-economic status classification was based on the occupation of main wage earner, namely: I (High) Professional, executive, managerial, technical; II (Middle) Semi-professional, e.g., nurse, teacher, clerks, sales; III (Low) Skilled, e.g., electrician, labourer, tradeperson; IV (Others) Unskilled, e.g., domestic duties, students, unemployed, retired.

## Statistical analysis

The results were analysed using the student's *t*-test, Fisher's Exact test, and regression tests, where appropriate, with Confidence Limits set at 95 per cent, and alpha value at 0.05. GraphPad InStat Statistical Package (Version 3.06, GraphPad Software Inc, San Diego, USA) was used for statistical analysis.

## RESULTS

Altogether, a total of 38 children were recruited into the study. Of these, 36 used the CHX gel for a period of three months, 33 for a period of six months, 25 for a period of nine months and 10 for a period of 12 months. These children had used the gel for varying periods of time because they were recruited at different time periods.

Table 1 shows the demographical characteristics of the 38 children (17 boys and 21 girls) who were infected with MS and given the CHX gel to brush on the teeth once daily. As shown in Table 1, there were no gender differences in the mean age, child care status, medical histories, toothbrushing frequencies and snacking frequencies. In spite of the standard recommendation that toothbrushing be performed by parents, 55 per cent reported self toothbrushing by the

**Table 1. Demography of children with *mutans streptococci* (MS) receiving chlorhexidine (CHX) treatment**

	Boys	Girls	Total†
n(%)	17 (45%)	21 (55%)	38 (100%)
Mean age at start of CHX (mths) ± SD	34.5±14.5	41.9±16.3	38.6±15.9
Socio-economic status			
I	4 (11%)	4 (11%)	8 (21%)
II	4 (11%)	6 (16%)	10 (27%)
III	8 (22%)	7 (17%)	15 (38%)
IV	1 (2%)	4 (11%)	5 (13%)
Principal childcarer			
Mother	13 (35%)	18 (47%)	31 (82%)
Father	2 (5%)	1 (3%)	3 (8%)
Both mother & father	2 (5%)	2 (5%)	4 (10%)
Childcare status			
Childcare & school	10 (27%)	17 (45%)	27 (72%)
None	7 (18%)	4 (10%)	11 (28%)
Medical history			
Nil significant	5 (13%)	6 (16%)	11 (29%)
Respiratory	4 (11%)	6 (16%)	10 (27%)
Reflux & GIT	2 (5%)	5 (12%)	7 (17%)
Others*	6 (16%)	4 (11%)	10 (27%)
Toothbrushing habits			
Daily frequency			
X1/less	8 (21%)	9 (24%)	17 (45%)
X2/more	9 (24%)	12 (31%)	21 (55%)
Self	8 (21%)	13 (34%)	21 (55%)
Parent assisted/supervised	9 (24%)	8 (21%)	17 (45%)
Snacks			
Sugar snacks	14 (37%)	15 (39%)	29 (76%)
Non-sugar snacks	3 (8%)	6 (16%)	9 (24%)
Frequencies (daily)			
1-2	7 (18%)	9 (24%)	16 (42%)
3 or more	10 (27%)	12 (31%)	22 (58%)

\*Other systemic diseases, disorders and/or infections.

†Differences in demographic characteristics between boys and girls are not statistically significant.

children. However, the daily application of CHX was performed by the parents, as revealed in the recall interviews.

Table 2 shows the effects of CHX on MS infection according to the compliance and period of use. Although the majority of children who became MS negative reported good or moderate compliance with chlorhexidine use at all time periods, the results reached statistical significance only at the six month time point ( $p=0.02$ ). Table 2 also shows the numbers and percentages of children who converted from positive to negative status at the three, six, nine and 12 month periods respectively. As seen in the Table, after three months 10 out of 36 (28 per cent) of the children tested negative for MS, while the other 26 children (72 per cent) continued to be positive. At six months, the total percentage of children who converted to negative MS had increased to 48 per cent (16/33). At nine months, 11 children became negative out of a total of 25 initially positive children (42 per cent). At 12 months, seven children converted to negative out of a total of 10 positive children (70 per cent). Correlation of the number of subjects who converted to negative at increasing time periods of three, six, nine and 12 months showed statistical significance (correlation coefficient,  $r=0.7331$ ) ( $p<0.039$ ). The chi-square value for trend was 5.121 ( $df=1$ ,  $p=0.024$ ).

Table 3 examines the oral hygiene habits, snacking frequency and mean daily sugar exposure of the children during the periods of CHX gel use. As shown in Table 3, at the three-month period, there were more children who converted to MS negative status who also reported the use of non-sugar snacks or no-snacking, compared to the group of children who continued to remain MS positive ( $p<0.039$ ). At six months, the mean daily sugar frequency was significantly higher in the children who continued to remain positive, compared to the group who became negative ( $2.11\pm0.74$  vs  $1.44\pm0.59$ ,  $p<0.007$ ). Differences in other factors between the groups such as oral hygiene habits did not reach significant levels (Table 3).

**Table 2. Effects of chlorhexidine (CHX) on *mutans streptococci* (MS) according to compliance and period of use**

Months on CHX		Total n (%)	Subjects who remained MS positive	**Subjects who converted to MS negative	p-value
3 months	Compliance	36 (100%)	26 (72%)	10 (28%)	n.s.
	Good-Moderate	29 (81%)	20 (57%)	9 (24%)	
	Poor	7 (19%)	6 (16%)	1 (3%)	
6 months	Compliance	33 (100%)	17 (52%)	16 (48%)	* $p=0.02$
	Good-Moderate	24 (73%)	9 (27%)	15 (46%)	
	Poor	9 (27%)	8 (24%)	1 (3%)	
9 months	Compliance	25 (100%)	14 (58%)	11 (42%)	n.s.
	Good-Moderate	18 (72%)	8 (32%)	10 (40%)	
	Poor	7 (28%)	6 (24%)	1 (4%)	
12 months	Compliance	10 (100%)	3 (30%)	7 (70%)	n.s.
	Good-Moderate	9 (50%)	3 (20%)	6 (30%)	
	Poor	1 (10%)	0 (0%)	1 (10%)	

\*Fisher's Exact Test.

ns: non-significant.

\*\*Correlation of number of subjects who converted to negative at various time periods showed statistical significance ( $r=0.7331$ ) ( $p<0.039$ ). The chi-square value for trend was 5.12 ( $df=1$ ,  $p=0.024$ ).

**Table 3. Effects of snacking and mean daily sugar frequency during the 3, 6, 9, 12 months periods**

Months on CHX	Oral hygiene habits and diet	Total n (%)	Subjects who remained MS positive	Subjects who converted to MS negative	p-value
3 months	<b>Toothbrushing habit (daily)</b>	36 (100%)	26 (72%)	10 (28%)	
	X1/less	20 (56%)	16 (44%)	4 (11%)	ns
	X2/more	16 (44%)	10 (28%)	6 (17%)	
	<b>Snacks</b>				
	No snacks	1 (3%)	1 (3%)	0 (0%)	
	Sugar snacks	25 (69%)	21 (58%)	4 (11%)	
	Non-sugar snacks	10 (28%)	4 (11%)	6 (17%)	p=0.03*
	<b>Mean sugar frequency (daily) ± SD</b>				
	Solution	1.56±0.81	1.56±0.87	1.57±0.67	ns
	Retentive	1.76±0.93	1.81±1.01	1.63±0.69	ns
Total	1.66±0.73	1.69±0.80	1.60±0.52	ns	
6 months	<b>Toothbrushing habit (daily)</b>	33 (100%)	17 (52%)	16 (48%)	
	X1/less	11 (33%)	7 (21%)	4 (12%)	
	X2/more	22 (67%)	10 (31%)	12 (36%)	ns
	<b>Snacks</b>				
	Sugar snacks	21 (64%)	13 (39%)	8 (24%)	
	Non-sugar snacks	12 (36%)	4 (13%)	8 (24%)	ns
	<b>Mean sugar frequency (daily) ± SD</b>				
	Solution	1.70±0.87	2.10±0.93	1.27±0.54	p=0.004**
	Retentive	1.88±0.98	2.13±0.99	1.62±0.93	ns
	Total	1.79±0.74	2.11±0.74	1.44±0.59	p= 0.007#
9 months	<b>Toothbrushing habit (daily)</b>	25 (100%)	14 (58%)	11 (42%)	
	X1/less	13 (52%)	8 (32%)	5 (20%)	
	X2/more	12 (48%)	6 (26%)	6 (22%)	ns
	<b>Snacks</b>				
	Sugar snacks	19 (76%)	11 (44%)	8 (32%)	
	Non-sugar snacks	6 (24%)	3 (14%)	3 (12%)	ns
	<b>Mean sugar frequency (daily) ± SD</b>				
	Solution	2.05±0.82	2.17±0.81	1.82±0.82	
	Retentive	1.98±0.72	1.86±0.71	2.03±0.85	
	Total	2.02±0.66	2.01±0.70	1.92±0.66	ns
12 months	<b>Toothbrushing habit (daily)</b>	10 (100%)	3 (30%)	7 (70%)	
	X1/less	1 (10%)	0 (0%)	1 (10%)	
	X2/more	9 (90%)	3 (30%)	6 (60%)	ns
	<b>Snacks</b>				
	Sugar snacks	8 (80%)	2 (20%)	6 (60%)	
	Non-sugar snacks	2 (20%)	1 (10%)	1 (10%)	ns
	<b>Mean sugar frequency (daily) ± SD</b>				
	Solution	1.37±0.60	1.44±0.51	1.33±0.67	ns
	Retentive	1.83±1.18	1.33±0.00	2.05±1.38	ns
	Total	1.60±0.78	1.39±0.25	1.69±0.93	ns

\*Chi-square=7.289, df=2, p<0.03.

\*\*t statistic=3.109, p<0.004.

#t statistic=2.864, p<0.007.

Post-treatment MS status of children after three, six and nine months is shown in Table 4. After three months post-treatment, out of a total of 29 children still in the study, 13 (45 per cent) children continued to be MS negative. These children had a history of good-moderate compliance of gel use (p=0.003). At six months post-treatment, 6/25 (24 per cent) children who continued to remain MS negative were also those who had good compliance of gel use. At nine months post-treatment, out of 15 children still in the study, three (20 per cent) children continued to be MS negative, while another 12 (80 per cent) remained MS positive (Table 4).

Toothbrushing habits and daily sugar frequency at the post-gel recall periods are shown in Table 5. Although the data suggests that those children who

converted to MS positive brush their teeth less frequently, and have higher mean daily frequencies of sugar snacks, the results did not reach statistical significance probably because of the small numbers in the groups.

## DISCUSSION

An important strategy for caries control in young children hinges on the removal of the cariogenic bacteria.<sup>1</sup> Although toothbrushing has been found to be clinically efficient in reducing their numbers,<sup>20</sup> removal of MS by oral antiseptics has additional benefits in the case of those children whose carers are unable to brush effectively. To date, only CHX and Povidone iodine have been identified as potential agents which are likely to be effective in removal of MS.<sup>8,21-25</sup> Povidone iodine

**Table 4. Effects of chlorhexidine (CHX) on *mutans streptococci* (MS) after cessation of CHX treatment according to compliance**

Months after CHX stop	Mean duration of CHX use (mths)		Total n (%)	Subjects who remained MS negative	Subjects who converted to MS positive/remained MS positive	p-value
3 months	9.3±2.5 mths	Compliance	29 (100%)	13 (45%)	16 (55%)	*p=0.003
		Good-moderate	21 (72%)	13 (45%)	8 (28%)	
		Poor	8 (28%)	0 (0%)	8 (28%)	
6 months	9.6±2.4 mths	Compliance	25 (100%)	6 (24%)	19 (76%)	n.s.
		Good-moderate	9 (76%)	6 (24%)	13 (52%)	
		Poor	6 (24%)	0 (0%)	6 (24%)	
9 months	10.4±1.9 mths	Compliance	15 (100%)	3 (20%)	12 (80%)	n.s.
		Good-moderate	10 (67%)	3 (20%)	7 (47%)	
		Poor	5 (33%)	0 (0%)	5 (33%)	

\*Fisher's Exact Test.

has been minimally researched because of its putative objectionable brown colour and reduced acceptance by children, as well as the contra-indications for patients with iodine hypersensitivity and thyroid pathosis. A few small studies reported that Povidone iodine has potential for decreasing MS and increasing the period of "disease-free state" in toddlers with high caries risk.<sup>23-25</sup>

On the other hand, CHX is well researched as a bactericidal agent against MS.<sup>8</sup> The cation chemical binds well to negatively-charged surfaces including bacterial cell walls, acquired pellicle, plaque layers and buccal mucosa.<sup>21</sup> As such, it has very good substantivity

and hydrophilicity which maximizes its antibacterial effects over an extended period of time. The long safety record and efficacy of CHX suggests a strong potential as an anti-caries agent.<sup>8,18</sup> However, previous investigations on the anti-caries effects of CHX have produced equivocal results. Those studies which were confined to adults, teenagers and older children, and using mainly CHX-containing varnishes, produced modest decreases in caries rates. A recent review mentioned that the majority of clinical trials on CHX were performed using CHX-containing varnishes, and results were inconclusive on the anti-caries effect for children and adolescents with regular fluoride exposure.<sup>22</sup> However, the same review found that four

**Table 5. Toothbrushing and mean daily sugar frequency during the post-gel recall periods**

Post-gel period	*Oral hygiene and dietary habits	Subjects who remained MS negative	Subjects who converted to MS positive
3 months	<b>Toothbrushing habit (daily)</b>		
	X1/less	3 (10%)	5 (17%)
	X2/more	10 (34%)	11 (38%)
	<b>Snacks</b>		
	Sugar snacks	8 (28%)	11 (38%)
	Non-sugar snacks	5 (17%)	5 (17%)
	<b>Mean sugar frequency (daily) ± SD</b>		
	Solution	1.42±1.09	2.04±0.73
	Retentive	2.06±0.94	2.11±0.87
	Total	1.74±0.77	1.93±0.64
6 months	<b>Toothbrushing habit (daily)</b>		
	X1/less	0	7 (28%)
	X2/more	6 (24%)	12 (48%)
	<b>Snacks</b>		
	Sugar snacks	3 (12%)	16 (64%)
	Non-sugar snacks	3 (12%)	3 (12%)
	<b>Mean sugar frequency (daily) ± SD</b>		
	Solution	1.06±0.93	1.52±0.72
	Retentive	1.72±0.71	2.21±1.40
	Total	1.45±0.77	1.65±0.94
9 months	<b>Toothbrushing habit (daily)</b>		
	X1/less	0	5 (33%)
	X2/more	3 (20%)	7 (47%)
	<b>Snacks</b>		
	Sugar snacks	2 (13%)	9 (60%)
	Non-sugar snacks	1 (7%)	3 (20%)
	<b>Mean sugar frequency (daily) ± SD</b>		
	Solution	0.78±0.69	1.20±0.91
	Retentive	1.78±0.69	2.60±1.11
	Total	1.28±0.48	1.90±0.76

\*During the post-gel recalls, there were no significant differences in oral hygiene and dietary habits in subjects who remained negative compared to those who converted to positive MS (p>0.1).

out of five studies which investigated the effect of CHX varnish on fissure caries were effective in preventing caries in children with low fluoride exposure.<sup>22</sup>

The present study was designed to determine the effectiveness of CHX as a treatment method for removal of MS from infected children who did not have dental caries. In the present study, removal of MS should be qualified to mean levels of MS which are not detectable by the microbiological test which are levels below 10<sup>3</sup> per ml, and are therefore likely to be of low clinical relevance.<sup>2,3</sup> The results showed that daily brushing with 0.2% CHX gel has the potential to remove MS in preschool children who have been infected with the bacteria. After three months of daily brushing with CHX, 28 per cent of children eliminated MS from the mouths to the extent that it could not be detected by the microbiological kit used. After six, nine and 12 months respectively, 48, 42 and 70 per cent of previously infected children tested negative. The increasing ability of elimination of MS with increasing time suggests that prolonged brushing of CHX gel is required for success.

However, in the consideration of efficacy, long-term post-treatment success rates should also be taken into account. Although the present results were significant for only the three-month post-gel recall period, it is noted that approximately 20 per cent of the children who were able to eliminate MS from their mouths with CHX brushing were able to prevent their re-appearance for nine months after stopping the gel. While it can be argued that this figure is low, the results demonstrate that a potential exists for CHX to eliminate MS in the long term. The authors hypothesize that this may be possible if other health behaviors are favourable as the oral microbial ecology of the young children is still immature and adaptable.

Despite study limitations, our results are thus in contrast to those of previous studies on older children and adults which have suggested that there is re-colonization after initial removal of MS by CHX.<sup>6,7</sup> In previous studies, inadequate removal of MS due to less intensive application may have caused the re-appearance of MS. This hypothesis is supported by previous studies which showed that the most persistent reduction of MS has been achieved using sustained release CHX varnishes.<sup>22</sup> In addition, the shorter duration of CHX treatment may be the reason for differences in results between the present study and those of Twetman and Grindejord who found re-colonization of MS in their toddler sample after three months.<sup>9</sup> In contrast to the two-week daily application of CHX in Twetman and Grindejord's study, the present results show that even with daily use of CHX, a period of at least six months is required for sustained removal of MS in preschool children.<sup>9</sup>

The effects of other variables, such as oral hygiene and sugar consumption which can also influence the removal of MS cannot be fully concluded from the present study. Although relatively small numbers are likely to have prevented the present results from

reaching statistical significance, a few trends are noticed, such as the clinical observation that CHX is more effective in those children who have better toothbrushing habits. In this regard, our findings are in line with those of Plotzitz *et al.* who stated that CHX cannot compensate for poor feeding habits and inadequate oral hygiene.<sup>13</sup> The effectiveness of toothbrushing in decreasing MS has also been demonstrated in a previous study which reported that there is elimination of MS from children when they commenced toothbrushing.<sup>20</sup>

Another trend which was noticed from analysis of post-treatment results was that less snacking frequencies were associated with less re-colonization of MS. Both these trends thus support previous reports that other good dental habits are important to prevent long-term re-colonization of MS.<sup>3,5,16</sup>

Although oral preparations of CHX used in high concentrations and frequency have been reported to cause adverse effects such as staining/discolouration of teeth, distorted taste, gingival bleeding, increased calculus epithelial desquamation<sup>22</sup> the subjects in the present study did not report any side effects. The lack of complications associated in children is likely to be related to microbial differences in the dental plaque of children or to salivary differences.

As the present study was designed as a follow-up clinical study of treatment cases of CHX, it lacks the advantages of a placebo-controlled trial. Furthermore, it demonstrates a major difficulty inherent in many longitudinal studies of young children, namely, the high mobility of families with young children which lead to high attrition rates which in turn reduces the subject numbers and accuracy of the results. A further limitation is the self-reported nature of the diet and toothbrushing data. However, the longitudinal nature of the study provides insight into the efficacy of MS removal by CHX gel using a simple and practical routine. In addition, follow-up of the children for over nine months after cessation of gel use has also provided interesting information regarding long-term benefits of the gel.

## CONCLUSIONS

This pilot study involving caries-free preschoolers with MS shows the potential of chlorhexidine gel to remove MS for the limited duration of study. The percentage of children who eliminated MS from their mouths increased from 28 per cent after three months, to 48 per cent after six months, and 70 per cent after 12 months of gel use. After stopping CHX for three months, 45 per cent of the children remained MS free. Regular toothbrushing and good diets should be used in conjunction with chlorhexidine to prevent re-colonization of MS in children.

## ACKNOWLEDGEMENTS

The present study was supported by the National Health and Medical Research Council of Australia and the Australian Dental Research Foundation. The

authors are grateful to Dr Harry Bartlett (Statistician, Department of Mathematical Sciences, Queensland University of Technology) for help with statistical analysis of data. The authors thank Colgate Oral Care (Australia) for its donation of Chlorhexidine Periogard® gel, toothbrushes and toothpaste used in the study, and all parents and children who participated in the study.

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