A Longitudinal Controlled Study of Factors Associated With Mutans Streptococci Infection and Caries Lesion Initiation in Children 21 to 72 Months Old

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Abstract

Purpose: The purpose of this longitudinal study was to determine factors associated with mutans streptococci (MS) infection and development of caries lesions in a group of children 21 to 72 months old.

Methods: The 63 caries-free children, recruited since birth, were divided into: (1) a study group of 24 children who were colonized with MS; and (2) a control group of 39 children without MS. The children were recalled every 3 months for approximately 24 months, and their social, medical, and dental histories were updated. At each recall, the teeth were checked for presence or absence of plaque, enamel hypoplasia, and caries lesions, and their MS status was assessed using a commercial test kit.

Results: MS infection was associated with: (1) visible plaque ($P < .01$); (2) enamel hypoplasia ($P < .05$); (3) commencement of tooth-brushing after 12 months of age ($P < .05$); (4) lack of parental assistance with tooth-brushing ($P < .025$); and (5) increased hours of child care/school ($P < .05$). Four children (20%) were colonized at an age range of 21 to 36 months, 9 (45%) at 37 to 48 months, and 7 (35%) at 49 to 72 months ($P < .001$). Eight children who developed caries lesions: (1) had more hypoplastic teeth ($P < .001$); (2) ate sugar-containing snacks ($P < .05$); and (3) did not brush regularly with chlorhexidine gel ($P < .01$) compared to those who remained free of caries lesions.

Conclusions: Lack of oral hygiene, consumption of sugar-containing snacks, and enamel hypoplasia are significant factors for both MS infection and caries lesion initiation. (Pediatr Dent 2006;28:58-65)

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Despite major advances in preventive dentistry in the past decades, dental caries remains one of the most significant oral conditions in young children. In preschoolers, early childhood caries (ECC) is highly prevalent among disadvantaged children and is a major cause of dental abscesses and toothache.[1]

Although the main etiological factors associated with dental caries—namely mutans streptococci (MS), sugar, and susceptible tooth surfaces—are well researched, many clinical issues important for prevention remain unknown. In particular, risk factors for caries in the youngest children have not been well studied. In this regard, longitudinal studies are of great value as they can provide information over defined periods of time. This helps to identify major factors in a child's dental, dietary, and oral hygiene histories in relation to the initiation and development of caries.[2-6]

In previous longitudinal studies, Wan et al (2001)[3] reported that MS could be detected in approximately 30% of babies as young as 3 months old. The main factors associated with MS colonization at the predentate stages were: (1) early weaning; (2) sleeping next to mother; (3) breast-feeding; and (4) consumption of sugar 2 to 3 times a day.[3] When the same children were followed-up after the teeth erupted, absence of oral hygiene was found to be another major factor encouraging MS colonization.[5] Thus, these previous results show that, upon eruption of the primary teeth, there are additional factors further influencing MS colonization.[5,6]

The aim of this study was, therefore, to report on further longitudinal follow-up of the same cohort of children to determine oral and general factors considered important in MS infection and development of caries lesions at the later ages of 21 to 72 months.

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Methods

Ethical approval for this study was obtained from the Human Research Ethics Committee of the University of Queensland, Brisbane, Australia. The children were recruited from birth as part a longitudinal study of MS colonization and recalled every 3 months.[3-5] Altogether, there were 63 children from the previous study available for recruitment into the present study. Their last report was undertaken at an approximate mean age of 24 months,[4] and the time lapse between the last report and the present investigation was approximately 6 months.

The dental follow-up visits were undertaken at the pediatric dentistry clinic of the School of Dentistry, University of Queensland, by a single examiner. Social and family histories of the children were taken to confirm previous records and update current conditions. Socioeconomic status of the children was obtained from the occupation of the family's main wage earner.[6] Medical and dental histories were taken to recheck neonatal histories and determine the presence of: (1) medical conditions; (2) past and present medications; (3) dental conditions; and (4) any dental treatment previously performed. Oral hygiene histories were taken to determine the frequency of daily tooth-brushing. All parents were informed regarding care of the child's teeth, including the fact that tooth-brushing should commence as soon as the teeth erupt. A 3-day prospective 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 were recorded in standard charts. The presence or absence of plaque on the primary incisors and canines was noted by visual examination.[18,19] Dental caries was charted using the World Health Organization (WHO) criteria.[7] Developmental enamel defects were recorded using the modified Developmental Defects of Enamel (DDE) index.[8] Briefly, enamel hypoplasia was recorded if there were surface defects in the form of pits, grooves, and areas of missing enamel. Enamel opacity was recorded if there were changes in the enamel's translucency.[8]

Intraexaminer consistency for recording of dental caries and enamel hypoplasia was performed using duplicate examinations of 20 children of similar ages from intraoral photographic records.[7] This method was used, as it would not be practical to perform detailed repeat examinations on toddler children on the same appointment to check for intraexaminer consistency.[7] The kappa statistic for intraexaminer consistency for the examiner using this method was found to be greater than 0.85.

At the commencement of the present study, the aforementioned data were collected and the children were divided into: (1) those with MS; and (2) those without the MS bacteria. To conform with ethical requirements, it was necessary to offer appropriate antibacterial treatment to the MS-infected group. Accordingly, all children who had detectable levels of MS were advised to brush daily with 2 drops with 0.2% chlorhexidine gel (Periogard, Colgate, Sydney, Australia) for a period of 12 months. The chlorhexidine gel was used after routine daily tooth-brushing with a child toothpaste (My First Toothpaste, Colgate, Sydney, Australia). The child's toothpaste contained sodium monofluorophosphate (MFP, 300 ppm fluoride ion). All parents of infected children consented to treatment with chlorhexidine gel. Children without the bacteria were the controls and were advised to perform routine daily tooth-brushing with a child's toothpaste only. Routine advice regarding diet and dental health was provided to all parents in both groups.

Test for MS infection

MS infection was determined using a commercially available microbiological test in which bacterial growth is semiquantitated on selective agar for MS (CRT Strip mutans test, Ivoclar, Mebourne, Australia).[9] Briefly, 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 adding 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 was assessed using reference charts provided by the manufacturer.[9]

Statistical analysis

The results were analyzed using Fisher's exact test, chi-square test, and analysis of variance (ANOVA) tests where appropriate, with confidence intervals set at 95% and alpha value at 0.05.

Results

At the time of entry into the present study, there was a total of 63 children (25 boys and 38 girls), and the mean age of the subjects was 35.5±14.9 months. The study group consisted of 39 MS-infected children. The other 24 children, who had undetectable levels of MS, constituted the control group. The results were analyzed for factors significantly related to the presence of MS at the commencement and at the end of the study period, respectively. The second part of the data analysis examined factors significant in the development of dental caries. Children were considered MS positive when 2 consecutive microbiological tests at 3 monthly intervals showed positive results. Thus, a child was considered positive at the first result if the second result was also positive.

Findings associated with MS infection at study's commencement (mean age=35 months)

Table 1 compares the demographic details and oral findings between the 24 children who had MS compared to the 39 others without MS. As shown in Table 1, no differences were noted in demographic characteristics, except for mother's mean age between MS-positive children (27.9±5.0 months) and MS-negative children (30.5±4.6 months; $P < .05$).

Table 1 also showed the intraoral findings associated with positive MS status at age 35 months. As shown in Table 1,

Table 1. Demographics and Oral Findings of Children With or Without Mutans Streptococci (MS) at Commencement of Study (Mean Age = 35 Months)

	MS subjects at age 35 mos	MS-negative subjects at age 35 mos	P value‡
n (%)	24 (38%)	39 (62%)	
Gender			
Male	11 (17%)	14 (22%)	
Female	13 (21%)	25 (40%)	NS
Mean age ± SD (mos)	35.6 ± 15.2 (9–73%)	35.4 ± 15.0 (14–82%)	NS
Mean gestational age ± SD (weeks)	38.3 ± 3.7 (27–42%)	38.2 ± 3.2 (30–42%)	NS
Mean birthweight ± SD (kg)	3.2 ± 0.9 (0.8–4.8%)	3.1 ± 0.8 (1–4%)	NS
Mean mother's age ± SD (ys)	27.9 ± 5.0 (15–34%)	30.5 ± 4.6 (22–43%)	< .05
Dental status			
Mean hypoplastic teeth per affected subject ± SD	9.5 ± 3.9	7.5 ± 6.0	< .05
Oral hygiene			
Plaque visible	10 (16%)	7 (11%)	
Plaque not visible	14 (22%)	32 (51%)	< .01
Tooth-brushing habits			
Tooth-brushing start			
Before 12 mos	14 (22%)	35 (56%)	
12 mos and after	10 (16%)	4 (6%)	< .05
Daily brushing frequency			
X1/less	14 (22%)	24 (38%)	
X2/more	10 (16%)	15 (24%)	NS
Self	15 (24%)	12 (19%)	
Parent assisted/supervised	9 (14%)	27 (43%)	< .025
Fluoride supplements			
Never	19 (30%)	31 (50%)	
Previously	3 (5%)	4 (6%)	
Current use	2 (3%)	4 (6%)	NS
Maternal MS status			
Positive	18 (29%)	14 (22%)	< .004
Negative	6 (10%)	25 (40%)	

the significant factors included:

1. mean number of hypoplastic teeth per child (9.5±3.9) in the MS-positive group vs (7.5±6.0) in the MS-negative group (P < .05);
2. plaque visible in anterior teeth (P < .01);
3. age of commencement of tooth-brushing (P < .05); and
4. whether parent assisted with tooth-brushing (P < .025).

Table 2 shows the subjects' dietary and feeding conditions at the initial examination (mean age=35.5±14.9 months). As shown in Table 2, significant findings regarding dietary and feeding included:

1. bottle-feeding present at 12 months old (P < .01);
2. solid food commencement at 6 months old (P < .05); and
3. use of sugar snacks (P < .01).

A habit of sleeping next to mother and early commencement of solid foods before 6 months of age were also significantly associated with MS-positive children at 35 months of age (P=.05).

Findings associated with MS infection at end of study (mean age=55 months)

As shown in Table 3, during the study, there were 8 (29%) children who remained consistently free of MS and another 20 (71%) children who became colonized. Table 3 also shows the general and oral health factors present in the groups of children with and without MS at the final examination at a mean age of 55 months.

Factors significantly associated with presence of MS were similar to those found at the initial examination (Table 3). These factors include:

1. attendance at child care centers and school (P < .05);
2. presence of plaque (P < .01); and
3. number of teeth affected by enamel hypoplasia (P < .025; Table 3).

Distribution of children at various colonization ages

Table 3 also shows the distribution of the children who were colonized by MS at 21 to 36 months old, 37 to 48 months, and 49 to 72 months, respectively. The differences in distribution of children colonized at these ages were statistically significant ($P < .001$).

Colonization ages and general and oral health factors

Table 3 also relates general and oral health factors to the distribution of the children colonized by MS at different ages. Attendance at child care center or school of over 20 hours per week was significantly associated with increased MS colonization at the later ages of 37 to 48 months and 48 to 72 months ($P < .05$).

Factors associated with caries development

At the end of the study, there was a total of 8 children who developed caries lesions, compared to another 27 who remained free of caries throughout the study period. Table 4 compares the general and oral health factors found in the group of children who developed caries lesions during the study period compared to the group which did not. As shown in Table 4, factors associated positively with caries lesion development included:

1. less tooth-brushing per day ($P < .025$);
2. use of sugar-containing snacks ($P < .05$);
3. high snacking frequencies ($P < .025$); and
4. presence of enamel hypoplasia ($P < .001$).

Compliance with the use of chlorhexidine gel is another factor which could have influenced the caries outcome. In the case of this variable, although there are missing data for 5 children in the noncaries group, the results suggest that caries is prevented with good compliance of chlorhexidine gel use (Table 4).

Correlation of MS colonization with caries lesion development

Table 5 correlates the age of colonization with the ages of lesions detection in the 8 children who developed caries lesions within the study period. Two children who were colonized at a mean age of 19.2±2.5 months developed caries lesions at a mean age of 37.0±1.4 months. There were another 3 children who were colonized at a mean age of 32.4±2.6 months who later showed caries lesions at a mean age of 45.3±11.8 months. Another 3 who were colonized at a mean age of 50.4±13.5 months showed caries lesions at a mean age of 60.3±2.5 months. The differences in ages of MS colonization and when caries lesions were detected were statistically significant, $P<.046$). Overall, the mean time difference between age of colonization and caries lesion development was 13.6±7.1 months. The mean number of teeth affected by caries lesions was 3.8±3.3.

Table 2. Dietary and Feeding Conditions of Children With and Without Mutans Streptococci (MS) at Commencement of Study (Mean Age=35 Months)

	MS subjects at age 35 mos	MS-negative subjects at age 35 mos	*P* value*
n (%)	24 (38%)	39 (62%)	
Feeding types			
6 mos and under			
Breast-fed	14 (22%)	23 (37%)	
Bottle-fed	4 (6%)	3 (4%)	
Bottle- and breast-fed	6 (10%)	13 (21%)	NS
7-12 mos			
Breast-fed	7 (11%)	15 (24%)	
Bottle-fed	12 (19%)	19 (30%)	
Bottle- and breast-fed	5 (8%)	2 (3%)	
No breast-/bottle-fed	0 (0%)	3 (5%)	NS
12 mos and after			
Breast-fed	3 (5%)	4 (6%)	
Bottle-fed	14 (22%)	35 (56%)	
Bottle- and breast-fed	2 (3%)	0 (0%)	
No breast-/bottle-fed	5 (8%)	0 (0%)	<.01
Sleep next to mother			
Yes and sometimes	8 (13%)	5 (8%)	
No	16 (25%)	34 (54%)	.05
Solid foods commence at:			
0-6 mos	23 (37%)	30 (48%)	
7 mos and later	1 (1%)	9 (14%)	< .05
Snacks			
Sugar snacks	19 (30%)	14 (22%)	
Nonsugar snacks	5 (8%)	25 (40%)	< .01

*NS=nonsignificant.

Discussion

The present study is a continuation of a longitudinal follow-up of a cohort of children recruited since birth. It also reports on significant factors associated with MS colonization and caries lesion development at later ages of the children. At commencement of the study, at the age of 35 months, the factors found to be positively associated with MS infection included: (1) mother's age; (2) enamel hypoplasia; (3) inadequate oral hygiene; (4) later age of commencement of

Table 3. Comparing General and Oral Health Factors of Mutans Streptococci (MS) -negative and MS-positive Children at End of Study (Mean Age=55 Months)

	MS-negative for entire duration of study n (%)	Became MS-positive during study n (%)	*P* value	Age (mos) at which subjects become MS positive N (%)			*P* value*
n (%)	8 (29%)	20 (71%)		21–36 mos (n = 4) (20%)	37–48 mos (n = 9) (45%)	49–72 mos (n = 7) (35%)	
Gender							
Male	1 (4%)	8 (30%)	NS	2 (10%)	4 (20%)	2 (10%)	NS
Female	7 (25%)	12 (41%)		2 (10%)	5 (25%)	5 (25%)	
Mean age (mos) ± SD							
At start of study	27.3 ± 10.2 (14–38%)	36.1 ± 15.2 (15–82%)	NS	21.3 ± 4.6	34.1 ± 7.5	47.0 ± 18.9	< .001
Became MS positive		48.3 ± 16.1 (21–28%)		28.0 ± 5.4	43.5 ± 3.7	65.9 ± 11.1	
Principal caregiver							
Mother	8 (29%)	17 (60%)	NS	4 (20%)	7 (35%)	6 (30%)	NS
Father	0 (0%)	2 (7%)		0 (0%)	1 (5%)	1 (5%)	
Both father and mother	0 (0%)	1 (4%)		0 (0%)	1 (5%)	0 (0%)	
Child care and School	5 (18%)	19 (68%)	< .05	4 (20%)	8 (40%)	7 (35%)	NS
None	3 (11%)	1 (3%)		0 (0%)	1 (5%)	0 (0%)	
Hours/week							
None	3 (11%)	1 (3%)	NS	0 (0%)	1 (5%)	0 (0%)	< .05
< 20	3 (11%)	7 (25%)		4 (20%)	2 (10%)	1 (5%)	
> 20	2 (7%)	12 (43%)		0 (0%)	6 (30%)	6 (30%)	
Oral hygiene							
Plaque visible	0 (0%)	11 (39%)	< .01	1 (5%)	6 (30%)	4 (20%)	NS
Plaque not visible	8 (29%)	9 (32%)		3 (15%)	3 (15%)	3 (15%)	
Tooth-brushing habits Daily frequency							
< 1	0 (0%)	7 (25%)	NS	1 (5%)	5 (25%)	1 (5%)	NS
≥ 2	8 (29%)	13 (46%)		3 (15%)	4 (20%)	6 (30%)	
Sweet snacks Frequencies (daily)							
1–2	3 (11%)	12 (43%)	NS	1 (5%)	6 (30%)	5 (25%)	NS
2–3	5 (18%)	8 (28%)		3 (15%)	3 (15%)	2 (10%)	
Dental status							
Mean hypoplastic teeth per affected subject ± SD	0	9.1 ± 3.08	< .025	10	12.3 ± 3.79	8.5 ± 0.71	NS
Maternal MS							
Positive	2 (7%)	10 (36%)	NS	2 (10%)	4 (20%)	4 (20%)	NS
Negative	6 (21%)	10 (36%)		2 (10%)	5 (25%)	3 (15%)	

*NS=nonsignificant; P>.1.

tooth-brushing; (5) lack of parental help with tooth-brushing; and (6) increased frequency of sugar snacks.

The results from previous investigations, together with the present data, show interesting age-related trends linked to the children's lifestyles. In the earliest period of childhood, when the child is physically dependent on the mother, the quantity of maternal MS is one of the most important factors influencing initial colonization of the child's mouth. The authors' first report on this cohort of children found that children whose mothers have the highest counts of bacteria were the earliest to acquire MS.[3-5]. These findings affirmed previous cross-sectional reports showing that the source of MS in children is usually the mother.[10-12] The present study also extends the authors' previous work to show that, as the children grew older, more became infected, and those who attended child care and school had increased chances of infection. This suggests that MS infection may also be acquired through increased socialization and contact with other individuals. This observation was noted at the end of the present study, when, at a mean age of approximately 55 months, maternal MS did not appear to influence the child's MS status.

Poor oral hygiene and plaque accumulation has been consistently shown to be a strong associating factor in ECC, and plaque control is likely to play a significant role in controlling MS after tooth eruption.[13-18] This hypothesis is supported by the authors' previous studies, which showed that commencement of tooth-brushing in toddlers can lead to the removal of MS in previously infected children when they begin tooth-brushing.[19]

Table 4. Comparisons of General Factors Between Children With and Without Caries Lesions at Age 4

Factors	Caries	Noncaries	*P* value*
n (%)	8 (22%)	27 (78%)	
Gender			
Male	4 (11%)	13 (37%)	
Female	4 (11%)	14 (41%)	NS
Socioeconomic status†			
I	0 (0%)	5 (14%)	
II–IV	8 (22%)	22 (64%)	NS
Tooth-brushing habit			
< 1 daily	6 (17%)	8 (23%)	
≥ 2 daily	2 (5%)	19 (55%)	< .025
By self	5 (14%)	11 (31%)	
Parents assisted/supervised	3 (8%)	16 (47%)	NS
Chlorhexidine use (compliance)‡			
Good to moderate	3 (10%)	18 (60%)	
Poor	5 (17%)	4 (13%)	< .03
Snacks categories			
Sugar snacks	8 (22%)	16 (47%)	
Nonsugar snacks	0 (0%)	11 (31%)	< .05
Snacks frequencies (daily)			
1–2	2 (5%)	20 (57%)	
≥ 3	6 (17%)	7 (21%)	< .025
Enamel hypoplasia			
No. (%) hypoplastic teeth which became carious	8 (6%)	0 (0%)	
No. (%) hypoplastic teeth which did not become carious	20 (16%)	102 (78%)	< .001

*NS=nonsignificant.
†Socioeconomic status classification based on occupation of main wage earner: I (high)=professional, executive, managerial, technical (eg, medical doctor, dentist, lawyer, accountant, engineer); II (middle)=semiprofessional (eg, nurse, teacher, clerks, sales); III (low)=skilled (eg, electrician, laborer, tradeperson); IV (others)=unskilled (eg, domestic duties, students, unemployed retired).
‡Compliance of chlorhexidine: good=use daily or miss 1x weekly; moderate=miss 2-3 weekly; poor=miss >3 weekly or hardly use.

The present study has also demonstrated that enamel hypoplasia is an important factor in MS colonization.[20-21] In this regard, children who are most susceptible to enamel hypoplasia—such as preterm and medically compromised children—are likely to be at highest risk for early MS colonization [21]

Although the sample of children who developed caries in the present study is small, the study's longitudinal nature has provided unique clinical value. The authors' data has demonstrated that there is a strong correlation between the timing of the child's MS acquisition and the development of the first caries lesions. Furthermore, although this study affirmed well-known associations of increased consumption of sugar snacks and poor oral hygiene with dental caries lesion development, it has proven on a longitudinal basis that enamel hypoplasia is an important caries risk factor. Additionally, the present study has also demonstrated that, in MS-infected children, daily tooth-brushing with 0.2% chlorhexidine gel can postpone the development of caries lesions.

Chlorhexidine, a commonly used and safe oral antimicrobial is well-known to be highly efficacious against MS and is likely to be useful for large-scale prevention of caries in children.[22-24] Placebo-controlled, double-blind studies

are currently being undertaken by the authors to explore the use of chlorhexidine for prevention of ECC.

In addition, the present study has demonstrated that the time span between MS colonization and caries lesion development is approximately 13 to 16 months. This duration of time should also be considered, taking into account the fact that the present cohort of children had been given 3 monthly recalls, oral hygiene instruction, dietary advice, and other preventive advice. It is reasonable to suggest that, in more high-risk children, the duration of time between MS acquisition and caries development is likely to be much shorter.

Although the present study has value as one of very few longitudinal studies on caries lesion development in children, the results need to be interpreted regarding their limitations. These include the loss of subjects to recall, which has resulted in relatively small numbers at the end of this study. Also, incomplete data in 5 of 30 children could have affected results of caries outcome with chlorhexidine use. Furthermore, the relatively low caries risk in the study children could have reduced the effects of the various influencing factors.

Conclusions

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

1. At a mean age of 35 months, MS infection is associated with:
   a. visible plaque in the mouth;
   b. later commencement of tooth-brushing (age 12 months or later);
   c. lack of parental assistance with tooth-brushing; and
   d. enamel hypoplasia,
2. At 55 months old, additional factors positively associated with MS infection included increased number of hours of child care/school.
3. The mean period of time between the detection of MS and caries lesions is approximately 13½ months.
4. Children who developed caries lesions had visible plaque, greater sugar frequency, less tooth-brushing and enamel hypoplasia compared to those who did not have caries lesions.
5. Children who remained free of caries were those who brushed regularly with chlorhexidine gel.

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Table 5. Correlation of Age of Mutans Streptococci (MS) Colonization With Caries Lesions Outcome

Age acquired MS (mos)	<24	24-36	≥ 37	Overall	P value*
No. of patients who have developed caries during period of study	2 (40%) (n=5)	3 (38%) (n=8)	3 (14%) (n=22)	8 (23%) (n=35)	NS
Mean age when caries first detected (mos)±SD	37.0±1.4 (n=5)	45.3±11.8 (n=8)	60.3±2.5 (n=22)	47.5±5.3 (n=35)	<.046
Time lapse between MS colonization and caries development (mos)±SD	17.8±1.1 (n=5)	12.9±9.3 (n=8)	9.9±10.9 (n=22)	13.6±7.1 (n=35)	NS
Mean no. of teeth affected by caries per affected subject±SD	2.5±2.1 (n=5)	5.7±4.6 (n=8)	3.3±3.2 (n=22)	3.8±3.3 (n=35)	NS

*NS=nonsignificant.

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