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COLONIZATION BY MUTANS STREPTOCOCCI IN THE MOUTHS OF 3- AND 4-YEAR-OLD CHINESE CHILDREN WITH OR WITHOUT ENAMEL HYPOPLASIA

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Summary—This case–control study compared the prevalence and concentration of mutans streptococci (MS) in saliva between children with and without enamel hypoplasia (EHP). A total of 486 3- or 4-year-old Chinese children were initially screened for EHP, then distributed into two groups: 234 children diagnosed as having EHP were assigned to the case group; 252 who were free of EHP were included in the control group. The concentration of MS in saliva was assayed for each child. Nutritional status was deduced from body height and weight. Birth weight, prematurity, and nursing history were also determined. MS were found in 94.7% of the study population. The differences in MS concentrations were not associated with low birth weight, prematurity, length of breast feeding, or body height and weight. A statistically significant association existed between the presence of EHP and high counts of MS ($p < 0.001$). High MS counts were correlated with severity of enamel defects ($p < 0.001$). When the caries status of the children was controlled as the confounding factor in statistical analyses, the association between EHP and MS decreased but still remained significant ($p = 0.025$). This study shows that high MS counts are correlated with EHP, suggesting that irregularities in enamel surfaces could be a contributing factor that fosters the increased colonization of MS in the mouths of children.

Key words: mutans streptococci, enamel hypoplasia, dental caries, children.

INTRODUCTION

The anatomical characteristics of the tooth are known to contribute to the initiation and progression of dental caries. Studies in animals show that under chronic malnutrition, enamel calcification is incomplete, resulting in delayed maturation of enamel at the time of tooth eruption (Pindborg, 1982; Sharawy and Yeager, 1991). For example, teeth that have not completely matured often exhibit fragmentary, coalesced, deep pits and fissures, particularly the first and secondary primary molars. These pits and fissures may provide sites for retention of cariogenic bacteria (Gibbons and van Houte, 1975). Accordingly, cavitation is more likely to occur on these altered tooth surfaces than on sound tooth surfaces.

Mutans streptococci are considered the primary cariogenic bacteria involved in dental caries. They are absent from the mouth of infants until after teeth emerge (Stiles *et al.*, 1976; Carlsson, Grahnen and Jonsson, 1975; Caufield, Cutter and Dasanayake, 1993). A number of factors in the oral environment have been related to colonization by mutans streptococci, including diet (in particular, a sucrose-rich diet), salivary composition, bacterial interactions, open caries lesions, eruption of teeth, and, possibly, oral hygiene (Emilson and Krasse, 1985; Gibbons, 1984; Gibbons and van Houte, 1975; Loesche, 1986). Pits, fissures and other retentive sites are more readily colonized by mutans streptococci than are smooth surfaces; this may account for their susceptibility to caries (Ikeda and Sandham, 1971; Svanberg and Loesche, 1977).

We speculated that children with enamel hypoplastic lesions might be colonized earlier by mutans streptococci, and have higher numbers of these bacteria in the mouth at a younger age than children who have normal enamel. Salivary concentrations of mutans streptococci reflect their overall colonization of the tooth surfaces (Emilson and Krasse, 1985; Jensen and Bratthall, 1989; Krasse, 1984), so we measured the concentration of mutans streptococci in the saliva of 3- and 4-year-old Chinese children to determine whether this was associated with enamel hypoplasia.

MATERIALS AND METHODS

Subject selection

Initially, we screened 1344 children, aged 3–5 years, for the presence of enamel hypoplasia. Children were recruited from two rural communities, Haidian and Miyun, within the Beijing metropolitan area of the People's Republic of China. Haidian, which is close to Beijing, has a higher economic development status than Miyun, which is approx. 100 miles away from Beijing. From this initial survey, the prevalence of enamel hypoplasia among the study population was found to be 22%. Two hundred and thirty-four 3- or

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Abbreviation: c.f.u., colony-forming units.

Table 1. Characteristics of the study population of Chinese children 3 to 5-years old by county, age, and gender

	Group				
	Children with enamel hypoplasis		Children without enamel hypoplasia		
	n	%	*n*	%	Total
Total	234	48.2	252	51.8	486
County					
Haidian	120	48.6	127	51.4	247
Miyun	114	47.7	125	52.3	239
Age (year)					
3	119	48.8	125	51.2	244
4	115	47.5	127	52.5	242
Gender					
Male	121	48.6	128	51.4	249
Female	113	47.7	124	52.3	237

4-year-old children exhibiting this condition were assigned as cases in a case–control study. Control subjects (252) were randomly selected from the total children who were free of the condition, using a random-digit table. Children in both groups were approximately equally represented according to age and gender (Table 1).

Assessment of enamel defects and dental caries

A modified index of developmental defects of dental enamel (DDE Index; Fédération Dentaire Internationale, 1982) was used to evaluate and rank the presence of enamel hypoplasia (Table 2). The enamel defect score described the severity of enamel defects for each subject. All buccal, lingual, and occlusal surfaces of each anterior and posterior tooth were examined for enamel hypoplastic lesions. After screening for such lesions, an examination for dental caries was given to all children, using the World Health Organization (WHO) standard criteria for dental caries diagnosis (WHO, 1987). If any tooth surface had a detectable softened area, the lesion was diagnosed as caries.

Assessment of mutans streptococci

The 'Strip mutans' test (Orion Diagnostica, Espoo, Finland) was used to estimate the concentrations of mutans streptococci in saliva. The children were asked to chew a piece of paraffin wax for 1 min and their saliva was then sampled as described by Jensen and Bratthall (1989). The strips were incubated in a selective medium containing bacitracin for 48 h at 37°C, then dried at room temperature. These strips can be stored at room temperature for long periods and the morphology of the attached colonies remains stable. The number of mutans streptococci per ml of saliva was estimated by comparing the density of colonies on the strips to a standardized chart. Four categories of bacterial density, (0, I, II, III), corresponded to 0, 1–10, 11–99 and >100 c.f.u. per strip, respectively. These categories reflected the approximate numbers of c.f.u. per ml of saliva at four concentrations, $<10^4$, 10^4–10^5, 10^5–10^6 and $>10^6$, respectively. The principal investigator (Y.L.) initially estimated the density of all the strip samples in the field. Strips were then shipped to Malmö, Sweden by Express Mail (4 days), where a second examiner scored each strip without awareness of the sample history. The interexaminer variation was then ascertained.

Table 2. Enamel defect score, criteria and codes of the modified DDE index used

Type of defect	Code
Normal	0
Opacity, all colours	1
Hypoplasia	
Pits	2
Diffuse grooves	3
Linear grooves	4
Missing enamel	5

The enamel defect score (EDS) for each individual is equal to:

$$EDS = \frac{\Sigma \text{ Criteria code} \times \text{No. affected tooth surfaces}}{\text{Total tooth surfaces at risk}} \times 10^*$$

*An arbitrary factor to reduce decimal figures.

Assessment of nutritional status

Nutritional status was determined by measuring the body weight and height of all the children. The Waterlow classification for degree of malnutrition to identify wasting and stunting (Waterlow *et al.*, 1977) and the WHO international standard (WHO, 1983) were used to classify them as normal or chronically malnourished.

Statistical analyses

All the data were organized and analysed using SPSS 5.0 statistical software package (Norusis/SPSS Inc., 1992). Descriptive and analytical techniques were used. The analysis of variance was used for comparison of the means of the enamel defect score with the strip-test results. The χ^2 and Mantel–Haenszel tests were used for comparison of the differences in the concentrations of mutans streptococci among subgroups. A probability value of <0.05 was considered statistically significant. A 95% confidence interval was used to compare different results within subgroups.

Table 3. Percentage distribution of the Chinese children (3 and 4 years old) for different concentrations of mutans streptococci

	Concentrations of mutans streptococci in saliva by categories*							
	0		I		II		III	
	n	%	*n*	%	*n*	%	*n*	%
Total	26	5.3	55	11.3	164	33.7	241	49.6
Enamel hypoplasia								
Absent	16	6.3	42	16.7	100	39.7	94	37.3
Present†	10	4.3	13	5.6	64	27.4	147	62.8
Dental caries								
Absent	5	9.1	11	20.0	21	38.2	18	32.7
Present†	6	3.0	15	7.6	58	29.4	118	59.9
County								
Haidian	8	3.2	23	9.3	90	36.4	126	51.0
Miyun	18	7.5	32	13.4	74	31.0	115	48.1
Age (year)								
3	11	4.5	26	10.7	75	30.7	132	54.1
4	15	6.2	29	12.0	89	36.8	109	45.0
Gender								
Male	13	5.2	30	12.0	88	35.3	118	47.4
Female	13	5.5	25	10.5	76	32.1	123	51.9

*Four categories of bacteral density, (0, I, II, III), corresponding to 0, 1–10, 11–99, and >100 c.f.u. per strip, respectively. These categories reflected the approximate numbers of c.f.u. per ml of saliva at four concentrations, $<10^4$, 10^4–10^5, 10^5–10^6 and $>10^6$, respectively (Bratthall and Carlsson, 1989).

†χ^2 test; $p < 0.001$.

RESULTS

Mutans streptococci were detected in 94.7% of the total study population. About 49.6% of children were in c.f.u. category III, while 33.7 and 11.3% were in c.f.u. categories II and I, respectively. To ensure that the interpretation of the code given to each individual was accurate, all the strips were re-examined by a second examiner. A 95.2% agreement was found between the two examiners, and the κ coefficient was 0.92, indicating a high interexaminer reliability.

The concentration of mutans streptococci in the saliva samples was significantly associated with the presence of enamel hypoplasia (Table 3). The proportion of children with low colonization of mutans streptococci (category I) was significantly higher for those without (16.7%) than with enamel hypoplasia (5.6%) ($p < 0.01$). The proportion of children with a high salivary concentration of mutans streptococci (category III) was twice as high (62.8%) in the group with than without enamel hypoplasia (37.3%) ($p < 0.01$). In Fig. 1, the percentage distribution of mutans streptococci among children with different types of enamel hypoplasia is illustrated. Of those with pit-type hypoplasia, 75.4% had high concentrations of mutans streptococci compared with 37.3% of the control group ($p < 0.01$). High concentrations were also seen in association with linear-type hypoplasia and missing enamel ($p < 0.01$).

The enamel defect score ranged from 0.4 to 31.9 among the total cases. Based on this scoring system, all cases were then reclassified into five groups.

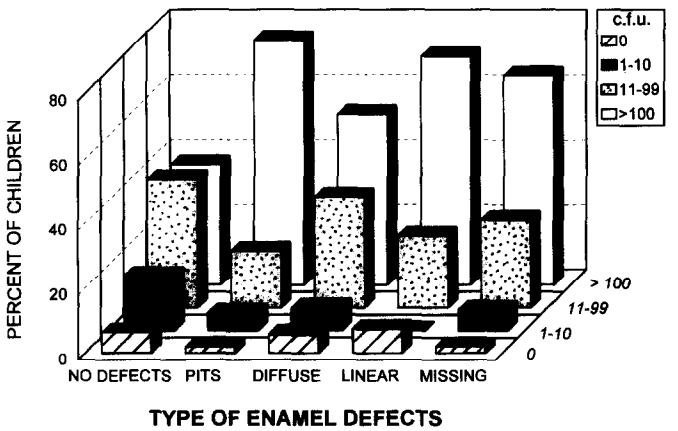

Fig. 1. Distribution of the number of c.f.u. of mutans streptococci among Chinese children (3- and 4-year-olds) showing different types of enamel defect (hypoplasia).

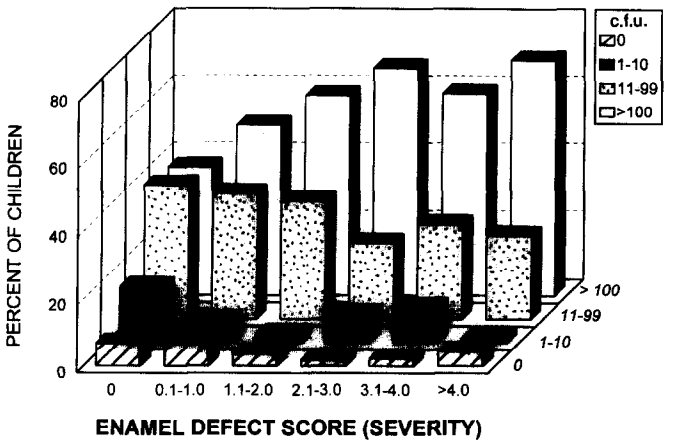

Fig. 2. Distribution of the number of c.f.u. of mutans streptococci among Chinese children (3- and 4-year-olds) with different severity (defined using the enamel defect score) of enamel hypoplasia on their tooth surfaces. The highest enamel defect score represents the most severe enamel defect.

Figure 2 shows that the proportion of children with a high number of mutans streptococci increased as the severity of enamel defects increased. By using the χ^2 test, the observed increases were found to be statistically significant ($p < 0.001$).

In addition to enamel hypoplasia, 84% of the children in the study population had caries on at least one tooth surface. Associations between the concentrations of mutans streptococci and caries, and between enamel hypoplasia and caries were spontaneously observed in this study (Table 3). To study the extent of the confounding effect caries had on concentrations of mutans streptococci, the Mantel–Haenszel and correlation analysis for controlling factors were used. The p-value for the correlation between the concentrations of mutans streptococci and enamel hypoplasia decreased from 0.001 to 0.025 after controlling for caries, but the results still remained statistically significant.

The concentration of mutans streptococci in saliva relative to age group, counties, and gender is also shown in Table 3. The proportions of children in each category were similarly distributed. No significant correlations were found between the number of mutans streptococci in saliva and low birth weight, limited breast feeding, prematurity, body height, or body weight (Table 4).

DISCUSSION

We sought to obtain cross-sectional information on the numbers of mutans streptococci in the saliva of 3- or 4-year-old Chinese children with or without enamel hypoplasia. Ninety-four % of the children harboured mutans streptococci, which is higher than in previous studies of similar age groups. For example, the prevalence of mutans streptococci among 2- to 5-year-old children was 75% in Sweden (Köhler, Andreen and Jonsson, 1988), 45% in Finland (Alaluusua *et al.*, 1990), 87% in Canada

Table 4. Comparison of the percentage distribution of the Chinese children (3 and 4 years old) for different concentrations of mutans streptococci by different nutritional measures

	Concentrations of mutans streptococci in saliva by categories							
	0		I		II		III	
	n	%	*n*	%	*n*	%	*n*	%
Birth weight (g)								
⩾2500	25	5.4	54	11.6	153	32.8	234	50.2
<2500	1	5.0	1	5.0	11	55.0	7	35.0
Prematurity								
No	25	5.5	50	11.1	154	34.1	223	49.3
Yes	1	14.3	1	14.3	1	14.3	4	57.1
Breast fed								
Yes	22	6.6	37	11.1	107	32.2	166	50.0
No	4	2.6	18	11.7	57	37.0	75	48.7
Height for age								
Normal	16	4.4	43	11.9	130	36.1	171	47.5
Stunted	10	7.9	12	9.5	34	27.0	70	55.6
Weight of height								
Normal	24	5.4	51	11.4	149	33.3	223	49.9
Wasted	2	5.1	4	10.3	15	38.5	18	46.2

None of the differences between values in this table was significant.

(Weinberger and Wright, 1989), and 85% in South Africa (Chosack *et al.*, 1988). The reported differences could be due to variations in assay methods, diet, salivary composition, or study population.

Our principal finding is that the prevalence and numbers of mutans streptococci in children's saliva were associated with the presence or absence of hypoplastic defects. Children with high enamel-defect scores were found to have high concentrations of mutans streptococci. Significantly, the children with the highest count were also the ones with the pit-type hypoplastic lesions. Samples of dental plaque taken from 'white spots' on tooth surfaces (slightly roughened, chalky areas) exhibit higher proportions of mutans streptococci than samples from sound enamel surfaces (Duchin and van Houte, 1978; Sansone *et al.*, 1993). Our results suggest that the anatomical characteristics of the enamel surface facilitate the colonization of mutans streptococci, and the type and number of teeth present are correlated with its initial acquisition (Caufield, Cutter and Dasanayake, 1993). Because the initial infection coincides with the emergence of molar teeth, and hence the presence of fissured occlusal surfaces, the morphology of tooth surfaces may play an important role in colonization by and accumulation of mutans streptococci.

An increase in salivary mutans streptococci correlates with the presence of dental caries (Bratthall and Carlsson, 1989; Hamada and Slade, 1980; Loesche, 1986). Because we found that the presence of enamel hypoplasia was significantly correlated with caries activity as well with increased concentrations of mutans streptococci in saliva, we were concerned that caries alone could account for the elevated levels of streptococci. To examine the contribution of enamel hypoplasia alone, irrespective of caries activity, we employed statistical tests in which caries activity was considered as a confounding variable, hence controlled. These analyses showed that increased concentrations of mutans streptococci remained significantly correlated with enamel hypoplasia. We interpret this finding to support the notion that enamel hypoplasia precedes both caries and elevated concentrations of the streptococci. Whether the increased caries associated with enamel hypoplasia leads to increased concentrations of mutans streptococci or whether the increased mutans streptococci concentrations due to enamel hypoplasia lead to increased caries cannot be determined from this cross-sectional study. Because rampant caries is frequently found in combination with enamel hypoplasia (Matee *et al.*, 1992), and children with rampant caries have appreciably more mutans streptococci than caries-free children (Loesche *et al.*, 1975), we suggest that hypoplastic lesions on enamel surfaces may contribute to the ability of mutans streptococci to colonize the tooth surfaces and, therefore, may result in a higher prevalence of dental caries.

From a nutritional point of view, our study showed that neither children's nursing history nor their nutritional status at birth or postnatally had a detectable influence on colonization by mutans streptococci. Furthermore, the lack of correlation between that colonization and low birth weight, limited breast feeding, or prematurity suggests that it is not dependent on the individual's general nutritional status. The majority of the children in our study population did not show severe malnutrition. Chronic malnutrition, particularly lack of protein and micronutrient deficiencies, may produce developmental impairment and degenerative changes in salivary glands and changes in saliva composition (Johansson *et al.*, 1992; Johnson, Sreebny and Enwonwu, 1977; Johnson and Alvares, 1984; Menaker and Navia, 1973; Watson and Antal, 1980). We did not evaluate salivary composition and its association with colonization by mutants streptococci. It is possible that a salivary effect could influence the high prevalence of mutans streptococci among the Chinese children studied.

In conclusion, we demonstrate significant differences in the degree of colonization by mutans streptococci in the mouth in children with and without enamel hypoplasia. The findings indicate that presence of mutans streptococci is associated with enamel hypoplasia, suggesting that irregularities and alterations on the enamel surfaces may promote its colonization.

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