Influence of colonization with mutans streptococci on caries risk in Japanese preschool children: 24 month survival analysis

Toshihiro Ansai, DDS, PhD   Asako Tahara, DDS, PhD   Masato Ikeda, PhD   Yasuhiro Katoh, DDS, PhD
Hideo Miyazaki, DDS, PhD   Tadamichi Takehara, DDS, PhD

Dr. Ansai is associate professor, Dr. Tahara is research fellow, Dr. Katoh is lecturer, and Dr. Takehara is professor at Kyushu Dental College, Department of Preventive Dentistry. Dr. Ikeda, is professor at University of Occupational and Environment Health, Department of Occupational Health Economics, Institute of Industrial Ecological Sciences, and Dr. Miyazaki is professor at Niigata University, School of Dentistry, Department of Preventive Dentistry, Japan. Correspond with Dr. Ansai ansai@kyu-dent.ac.jp

Abstract

Purpose: This study evaluates how various microbial- and salivary-related risk factors influenced the hazard for caries development in preschool children.

Methods: The study population consisted of 131 subjects (age: 0.5 to 6.0 yrs).   Oral examination, including two bacterial tests and buffering capacity test, was conducted at six month intervals over 24 months.   A survival analysis was used to describe caries hazard over a 24-month follow-up period.   A Cox proportional hazards regression analysis was performed to test the influence of salivary mutans streptococci (MS), aciduric bacteria, buffering capacity and age on caries development.

Results: Of the total subjects, 60 children (46 %) were found to be caries-free at baseline.   Caries hazard correlated significantly with salivary MS levels at baseline (relative risk, 1.7; P= 0.003), but not with aciduric bacteria and buffering capacity.   This analysis showed that all of children with high colonization of MS at baseline had dental caries 15 months later.

Conclusion: The results suggest that salivary MS level at baseline influenced caries hazard in preschool children. (Pediatr Dent 22:377-380, 2000)

The World Health Organization (WHO)[1] and Fédération Dentaire Internationale (FDI)[2] report that one of the global goals concerning oral health in the years 2000 is, "the prevalence of caries in children 5- to 6-year-old should fall to less than 50%".   However, in a national survey by the Dental Health Division of the Japanese Ministry of Health and Welfare[3] conducted in 1993, the prevalence of primary dental caries was higher with increasing age among 2 to 4 year-old children, and reached a high of 77% in 5 year-olds.   Moreover, in the 1995 WHO international database,[4] 12 year-old Japanese children had higher DMF scores than those of children in other developed countries.   Therefore, in Japan there is an urgent need for the establishment of an effective preventive program of dental caries for preschool children.

Caries risk tests often used for clinical purposes include flow rate of saliva, buffering capacity, growth of colony-forming microorganisms, counts of mutans streptococci (MS), and bacterial aciduric and acidogenic ability.[5]   Previous studies have shown a close relationship between such tests and incidence of dental caries in preschool children.[6, 7]   Furthermore, socioeconomic variables, such as social class, immigrant status, and mother's education have been also shown to be important risk factors.[8, 9]

On the other hand, based on literally hundreds of laboratory and clinical observations,[10-12] the MS have the necessary chracteristics to induce dental caries, including various acid producing and acid tolerance chracteristics.   According to the report by Caufield *et al.*,[13] MS are transmitted vertically, during a discrete time period between 19 and 33 months of age.   To date, there are several reports of longitudinal studies that examined the influence of potential caries risk factors at baseline on caries development in preschool children.[9, 14, 15]

The objective of the present study was to evaluate how the individual's baseline caries risk, or MS, aciduric bacteria, and buffering capacity, has an influence on the hazard for caries development in children from 0.5 to 6 years of age.   For this purpose, 60 caries-free children at baseline were monitored every 6 months during a 2-year period.   The results of a Cox proportional hazards regression analysis, which was used to analyze the "survival" time until caries development are reported.   Such analysis has not been used previously in this field.

Methods

Population

The study was carried out in Kitakyushu city (where drinking water is not fluoridated) with a population of 1,000,000.   A total of 131 Japanese children from one kindergarten in Kitakyushu city, aged 0.5 to 6.0 years (mean age at entry into study, 3.5 years), were examined for dental caries and sampled for oral bacteria including MS.   These tests were performed at entry and twice annually for 24 months.   The average dfs (decayed, filled surfaces of primary teeth) in this sample was 4.66.   At the time of the study, there were no specific oral health programs in this community.   The procedures, possible discomforts or risks, as well as possible benefits were explained fully to the children, and their parents' informed consent was obtained prior to the investigation.

Caries risk tests

Testing for caries risk was conducted using commercially available kits. These included the Dentocult SM®, Dentobuff® (Orion Diagnostica, Espoo, Finland), and Cariostat® (Sankin, Japan) tests. All tests were performed according to the instructions provided by the manufacturer. Dentocult SM® is a dip slide test for evaluation of MS counts for clinical use. In this study, Dentocult SM® was used for the detection of MS using stimulated saliva samples, although unstimulated saliva was used in children under 2 years of age since they can not use the paraffin wax required for obtaining stimulated saliva. Prior to the investigation, to confirm the effectiveness of unstimulated saliva in the Dentocult SM® system, unstimulated saliva and plaque in 48 children under 2 years of age were sampled and analyzed. A significant agreement (90 %) and kappa value (0.79, *P*<0.01) between the MS levels of each sample was observed. After incubation for 2 days at 37°C, MS levels were estimated from the comparison scale provided by the manufacturer. The scores were categorized and assigned caries risk as follows: not detectable, $< 10^4$ CFU/ml; low, $\geq 10^4$ to $<10^5$ CFU/m; moderate, $\geq 10^5$ to $< 10^6$ CFU/ml; high, $\geq 10^6$ CFU/ml.[16]

Buffering capacity was estimated from unstimulated whole saliva collected from the floor of the mouth using a Dentobuff®.[17] The Cariostat® test uses a high concentration of sucrose solution to evaluate the acidogenic ability from sucrose of the aciduric bacteria in dental plaque.[5] Plaque was collected from the buccal surfaces of all maxillary teeth using a sterilized cotton swab supplied in the kit, transferred into a test medium in a Cariostat® test tube and incubated at 37°C for 48 hours. Changes in color of the test media were compared with the four reference tubes provided in the test kit. The readings of the Dentocult SM®, Dentobuff® and Cariostat® tests were performed by one trained examiner (AT) who was unaware of the results of dental examinations.

Oral examination

Dental examination of each subject was conducted in the kindergarten under sufficient illumination, using a plane dental mirror and a dental probe (YDM Yamaura, Japan). The presence of dental caries was based on the criteria established by WHO.[1] Dental caries experience was expressed as the sum of decayed and filled surfaces of deciduous teeth (dfs) for each subject. Dental examinations were carried out by two dentists (YK and TA), who were blinded to the data of caries risk tests, at six month intervals from April, 1992 (baseline) to April, 1994 (final examination). To confirm the reliability between examiners, a duplicate examination was performed on 30 randomly selected children before these examinations. The results showed no significant disagreement in dfs scores between the two dentists (92% agreement, kappa = 0.83).
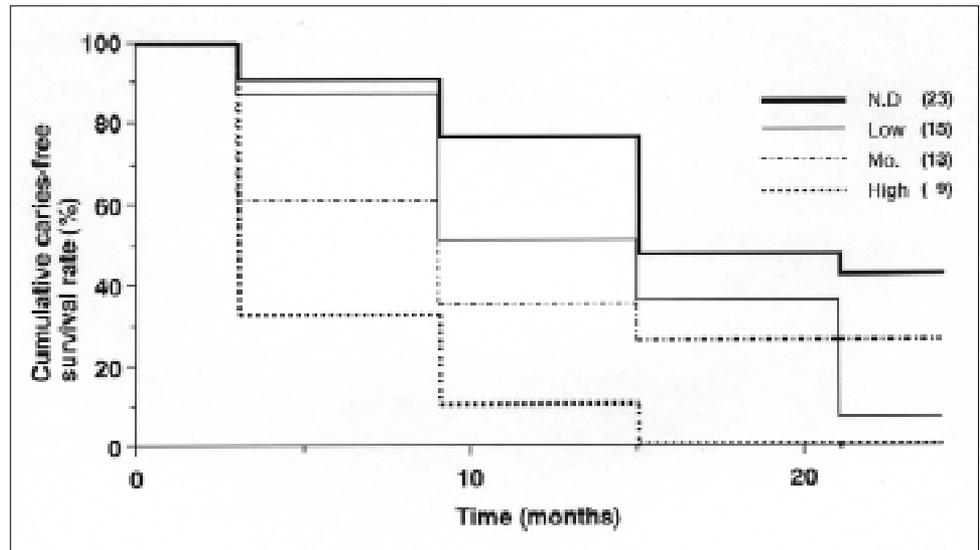

Fig 1. Cumulative survival curves of caries hazard for 60 caries-free children, based on the MS level at baseline (Kaplan-Meier estimations), (The number of subjects in each level is shown in parenthesis.) N.D=not detected.

Data analysis

In order to evaluate the reliability of the diagnosis of dental caries, kappa statistical test was used for testing intra- and inter-examiner reliability of detecting dental caries.

The association between the hazard for dental caries development and the risk factors of dental caries was evaluated by the survival analysis as follows. The time of end point was determined when the first dfs (decayed, or filled surfaces) was detected in primary dentition. The detection month was determined by subtracting 3 months from the last examination month for the child concerned, with the allowable margin of error within 3 months at most. Based on the level of MS, aciduric bacteria and buffering capacity, the cumulative caries-free survival rates among the children were calculated according to the method of Kaplan-Meier.[18] The Cox proportional hazards model[18] was used to assess the significance of excess risk of the risk factors and to determine the hazard ratios (relative risk) and 95 percent confidence intervals. The explanatory variables included in the multivariate Cox regression analysis were MS level, aciduric bacteria level, buffering capacity, and age. All P values of less than 0.05 were considered to indicate statistical significance. The analysis was performed using SAS™ software for windows.

Results

At baseline in this study, the prevalence of salivary MS colonization in 0.5- to 1.4-year-olds (N=14) and 1.5- to 2.4-year-olds (N =24) was 23% and 49%, respectively, and reached $\geq 80\%$ in 3.5- to 6.0-year-olds children (N=71). Of the total 131 preschool children, 60 (46%) were found to be caries-free children at baseline. The breakdown of subject age was 0.5- to 1.4-year-olds (N=14), 1.5- to 2.4-year-olds (N=20), 2.5- to 3.4-year-olds (N=13), 3.5- to 4.4-year-olds (N=6), 4.5- to 5.4-year-olds (N=5), and 5.5- to 6.0-year-olds (N=2), respectively. Since baseline was April, 1992 and the last examination was April, 1994, the follow-up period was 24 months. In the present study, caries-free children at baseline were selected in order to observe the hazard for caries development during 24 months. This study was designed to examine the children before they enter elemen-

Table 1. Assessment of Caries Risk Tests at Baseline in 60 Caries-Free Children					
			Risk		
Variable	N.D[*]	Low	Moderate	High	% at Risk
Mutans streptococci	23	15	13	9	62
Aciduric bacteria	12	38	10	0	80
Buffering capacity	11	27	22	—[**]	82

[*] N.D=not detected.

[**] The kit of buffering capacity does not have the corresponding score.

tary schools at age 7. Therefore, although 7 children reached the age of 7 during the follow-up period, these were not considered as the follow-up loss. Also, only 3 children (before 4-year-olds at baseline) were lost during the follow-up period—as a result of moving to another kindergarten. The mean age at baseline of 60 caries-free children was 2.6 years (range, 0.5 - 6 years), the median socio-economic level was middle class. Table 1 shows the results of assessment of caries risk tests in caries-free children at baseline. The percentages of children at risk according to MS, aciduric bacteria and buffering capacity were 62, 80, and 82%, respectively.

Table 2 shows the results of the Cox proportional hazards regression analysis. Only MS level was statistically significant, but not other factors including age as one of the confounding factors. The relative risk for MS was positive, indicating a higher hazard rate of caries development with high MS levels compared to low MS levels.

Figure 1 shows cumulative caries-free survival rate at follow-up computed for various MS levels. Approximately 90% of children with high MS level at baseline had dental caries 9 months later, while only 23% children with not detected level of MS at baseline developed caries at the same period.

Discussion

In the present study, the survival analysis we used to investigate the relationship between putative caries risk factors and caries hazard over a 24-month period. Findings suggested that MS was the predictive of caries risk, but not aciduric bacteria and buffering capacity. The final goal is to obtain basic data to establish a preventive program of dental caries for preschool children. In general, community oral health programs such as water-fluoridation might be performed at first, but water-fluoridation is not yet acceptable in Japan. Therefore, in any longitudinal study, it is important to identify the most appropriate caries risk factors. In this regard, Powell [9] recently reviewed various caries prediction models in children with primary dentition. According to this review, significant factors include past caries experience of primary incisor caries, bacterial levels of MS, and sociodemographic variables such as immigrant background and sugar intake. The results in this study were consistent with the reports included in the above review that MS colonization levels positively correlated with caries development, and support the notion that MS is the best predictor of future caries development in primary dentition.

On the other hand, little information is available on colonization of salivary MS in young Japanese children aged < 6 years of age. Fujiwara et al.[19] investigated the prevalence of salivary MS colonization in < 2-year old Japanese children and reported that 7% and 20% of children showed colonization in 0.5-1.0 year old and 1.0 to 1.5-year olds, respectively. The present data of MS colonization from 131 children (23% in 0.5-1.4 year-old children) are almost similar to those of Fujiwara et al.,[17] thus indicating that this age is the time of initial infection among young Japanese children. Interestingly, the prevalence of salivary MS colonization in Japanese preschool children described here is also in agreement with the data reviewed by van Palenstein Helderman et al.,[20] which showed that the prevalence of salivary MS reached ≥ 80% in children aged 3.5 to 6.0 in most countries of the world. Our results thus strongly support their data and others that MS is ubiquitous among children aged 3.5 years or older with a complete primary dentition. Furthermore, in the study, approximately 20% of children older than 3.5 years of age did not show MS colonization. Caufield et al.[13] reported that 17% of children were free of detectable levels of MS throughout the study period of five years. Thus, it seems reasonable to speculate that the proportion of children which is resistant to MS colonization might increase in developed countries where "rampant caries" has already declined. It is hoped that the number of such children might gradually increase in the future.

On the other hand, in the present study, the proportion of children with caries was around 60% during the first two years in children without MS at baseline (Fig 1). However, almost all of these children were infected with MS during the follow-up periods. Conversely, several children were still caries-free during two years, although they were infected with MS at a low level during the follow-up periods. In this respect, it would be important to bear in mind that the method used in the present study has a low sensitivity for detecting very low levels of MS. Moreover, some studies[16, 21] showed that the Dentocult SM. system alone was not efficient in selecting persons at high risk for caries (high specificity [88%], but low sensitivity [30%]). Therefore, there might be limitations of the usefulness of this method to accomplish the goals of the program to identify subjects at risk.

Assuming that the initial colonization by MS influenced the outcome (caries development) as indicated above, it is important to design effective strategies to prevent the development and progression of caries in individual children once high MS colonization is noted. These results suggest that clinical intervention strategies should be included in preventive programs for dental caries.

In this study, a Cox proportional hazards regression analysis provided important information, although a limited number of subjects and variables were investigated. This study has implications for caries prediction study designs and public preventive programs for dental caries. A longitudinal study using a larger population sample to complement the results of the present study is planned.

Table 2. Cox Proportional Hazards Analysis for Caries Development (N=60)		
Variable	Relative Risk (95% Cl)	P value
Mutans streptococci	1.7 (1.18-2.32)	0.003
Aciduric bacteria	1.4 (0.79-2.52)	0.243
Buffering capacity	1.2 (0.72-1.95)	0.505
Age	1.0 (0.72-1.30)	0.818

Conclusion

A Cox proportional hazards regression analysis showed that caries hazard correlated significantly with salivary MS levels at baseline. These results suggest that once children show colonization of MS in the oral cavity, they are likely to develop caries of primary teeth. This data is useful for designing studies to assess effective caries preventive strategies including clinical intervention to reduce colonization with MS.

References

1. World Health Organization: Oral Health Surveys, Basic Methods, 3rd edn. Geneva: World Health Organization, 1988.
2. Fédération Dentaire Internationale: Global goals for oral health in the year 2000. Int Dent J 32: 21-28, 1985.
3. Dental Health Division of Health Policy Bureau Ministry of Health and Welfare Japan: Report on the survey of dental disease (1993). Tokyo: kokuhokenkyokai, pp 43-44, 1995 (in Japanese).
4. World Health Organization: Dental caries levels at 12 years. Geneva: World Health Organization, 1996.
5. Camling E, Emilson CG: Results with the caries activity test "Cariostat" compared to prevalence of mutans streptococci and lactobacilli. Swed Dent J13: 125-130, 1989.
6. Bretz WA, Djahjah C, Almeida RS, Hujoel PP, Loeche WJ: Relationship of microbial and salivary parameters with dental caries in Brazilian pre-school children. Community Dent Oral Epidemiol 20: 261-264, 1992.
7. Thibodeau EA, O'Sullivan DM, Tinanoff N: Mutans streptococci and caries prevalence in pre-school children. Community Dent Oral Epidemiol 21: 288-291,1993.
8. Wendt LK, Jonsell R: Illness and use of medicines in relation to caries development and to immigrant status in infants and toddlers living in Sweden. Swed Dent J 20: 151-159, 1996.
9. Powell LV: Caries prediction: a review of the literature. Community Dent Oral Epidemiol 26: 361-371, 1998.
10. Krasse B, Jordan HV, Edwardsson S, Svensson I, Trell L: The occurrence of certain "caries inducing" streptococci in human dental plaque material with special reference to frequency and activity of caries. Arch Oral Biol, 13:911-918, 1968.
11. Loesche WJ: Role of *Streptococcus mutans* in human dental decay. Microbiol. Rev, 50:353-380, 1986.
12. Tanzer JM: Essential dependence of smooth surface caries on, and augmentation of fissure caries by, sucrose and *Streptococcus mutans* infection. Infect Immun, 25:526-531, 1979.
13. Caufield PW, Cutter GR, Dasanayake AP: Initial acquisition of mutans streptococci by infants: evidence for a discrete window of infectivity. J Dent Res 72: 37-45, 1993.
14. Grindefjord M, Dahllöf G, Modéer T: Caries development in children from 2.5 to 3.5 years of age: a longitudinal study. Caries Res 29: 449-454, 1995.
15. O'Sullivan DM, Thibodeau EA: Caries experience and mutans streptococci as indicators of caries incidence. Pediatr Dent 18 (5): 371-374, 1996.
16. Alaluusua S, Kleemola-Kujala E, Grönroos L, Evälahti M: Salivary caries-related tests as predictors of future caries increment in teenagers. A three-year longitudinal study. Oral Microbiol Immunol 5: 77-81, 1990.
17. Grindefjord M, Dahllöf G, Ekström G, Höjer B, Modéer T: Caries prevalence in 2.5-year old children. Caries Res 27: 505-510, 1993.
18. Lee ET: Statistical methods for survival data analysis. New York: John Wiley & Sons, 1992.
19. Fujiwara T, Sasada E, Mima N, Ooshima T: Caries prevalence and salivary mutans streptococci in 0-2-year-old children of Japan. Community Dent Oral Epidemiol 19: 151-154, 1991.
20. Van Palenstein Helderman WH, Matee Min, Van der Hoeven, Mikx FHM: Cariogenicity depends more on diet than the prevailing mutans streptococcal species. J Dent Res 75: 535-545, 1996.
21. Emilson C-G, Krasse B: Comparison between a dip-slide test and plate count for determination of *Streptococcus mutans* infection. Scand J Dent Res 94: 500-506, 1986.

ABSTRACT OF THE SCIENTIFIC LITERATURE

CHLORHEXIDINE VARNISH TO PREVENT GINGIVITIS WITH ORTHODONTIC PATIENTS

The aim of this study was to investigate the effects of a chlorhexidine/thymol containing varnish on the levels of four specific bacteria in the gingival fluids of 15 adolescents undergoing fixed orthodontic treatment who had mild chronic gingivitis. A split mouth study design was used, using either the test varnish (Cervitec) or a placebo varnish. The varnish was allowed to set for 20-30 sec. and no brushing for 24 hours. The patient returned at 3, 8, and 30 days after baseline. A significant reduction of the tested bacteria occurred suggesting that Cervitec Varnish is beneficial in patients with chronic gingival inflammation.

Comments: Chlorhexidine varnish has an important role to help control orthodontic patients with mild gingivitis for up to 30 days. Longer follow up is needed to determine when one needs to repeat the application of the varnish. **LHS**

Address correspondence to: Tulay Yucel-Lindberg, Dept. of Pediatric Dentistry, School of Dentistry, Karolinska Institutet, Box 4064, SE-141 04, Huddinge, Sweden.

Effect of an antibacterial dental varnish on the levels of prostanoids, leukotriene B4, and interleukin-1B in gingival crevicular fluid. Yucel-Lindberg, T., Twetman, S., Skold-Larsson, K., and Modeer, T. Acta Odontol Scand 57(1):23-27, 1999.

22 references