Association of Maternal–Child Characteristics as a Factor in Early Childhood Caries and Salivary Bacterial Counts

Nazan Kocatas Ersin, PhD, DDS Nesrin Eronat, PhD, DDS Dilsah Cogulu, PhD, DDS Atac Uzel, PhD Sadik Aksit, MD

ABSTRACT

The aim of this study was to investigate the caries-related microorganisms in saliva and the prevalence of Early childhood caries (ECC) in 15- to 35-month-old Turkish children and their associations with the characteristics of the mothers, socioeconomic criteria, and feeding habits of the children.

Methods: Saliva samples of 101 children were studied to determine the numbers of *Streptococcus mutans*, lactobacilli, and *Candida albicans.* A questionnaire regarding the characteristics of the mothers, socioeconomic criteria, and feeding habits of the children was carried out before the dental examinations of the mother-child pairs.

Results: The regression analyses revealed DMFS scores of the mothers as an impact factor for the children's caries experience. The prolonged usage of feeding bottle with sweetened milk, pacifier use, and maternal sharing were strongly associated with the colonization of *S mutans*, lactobacilli, and *C albicans*, respectively. A significant correlation was also found between maternal education and *S mutans*.

Conclusion: Data indicated that the mother's DMFS scores, education, and feeding habits were strong risk indicators for the colonization of caries-related micro-organisms and ECC. (J Dent Child 2006;73:105-111)

KEYWORDS: EARLY CHILDHOOD CARIES, MATERNAL, *STREPTOCOCCUS MUTANS*, LACTOBACILLI, *CANDIDA ALBICANS*, FEEDING HABITS

Early childhood caries (ECC) is a specific form of rampant caries that initially affects the primary maxillary anterior teeth of infants and young children. As the disease progresses, decay appears on the occlusal surfaces of the primary maxillary first molars, with subsequent spread to other primary teeth, resulting in the eventual destruction of the primary dentition.[1]

ECC is an infectious disease where microorganisms play a major role and mutans streptococci (MS) are believed to be the major etiological agents of tooth decay. Children are more likely to acquire MS in their oral flora primarily from their mothers due to the frequent maternal postnatal contact during the window of infectivity, which is around 2 years of age. Thus, it has been reported that there is a quantitative correlation between the levels of MS in mothers and their children. This suggests that, by identifying mothers-to-be with high MS levels and intervening to optimize the mother's oral health so as to reduce MS while the mother is still pregnant, it may be possible to reduce the incidence of caries affecting young children. Even though high levels are closely associated with caries risk, MS is a normal inhabitant of the mouth. Furthermore, high MS, by itself, is not necessarily a reliable predictor of caries risk. Results suggest, however, that a child's MS level, combined with other indicators, could be predictive of a child's caries risk.[2-4]

Dr. Ersin is research assistant, Dr. Eronat is professor, and Dr. Cogulu is research assistant, Department of Pedodontics, Faculty of Dentistry, Ege University, Bornova–Izmir, Turkey; Dr. Uzel is research assistant, Department of Biology Basic and Industrial Microbiology, Faculty of Science, Ege University, and Dr. Aksit is professor, Department of Pediatrics, Faculty of Medicine, Ege University.
Correspond with Dr. Ersin at nazan.ersin@ege.edu.tr

Some studies of caries risk factors in children have attempted to combine children's MS with other variables to predict caries susceptibility in children. Among the variables investigated have been children's diet, oral hygiene, oral habits, and mother's demographic data. Diet plays a critical role in acquisition of the bacterial infection and ECC development. Experimental dietary studies in humans and laboratory animals have established that the frequency of intake of fermentable carbohydrates, especially sucrose, can significantly influence the plaque and salivary levels of MS, lactobacilli, and yeasts. Feeding habits—such as prolonged usage of a feeding bottle, sweetened pacifiers, frequent intake of sugar-containing snacks, and prolonged breast-feeding—also enhance caries development in children. Furthermore, unsatisfactory feeding habits—such as bottle-nursing with a sweetened liquid, especially during sleep—intensifies the risk of disease in children, as oral clearance and salivary flow rate are decreased. [5,6] Poor oral hygiene is also considered to be one of the major risk factors in ECC. It is suggested that brushing twice a day at an early age with parents could avoid the risk of caries.[7,8]

In addition, characteristics related to mothers—such as high MS levels, high caries experience, poor oral hygiene, low socioeconomic status, and low education levels—could be risk indicators of caries for their children.[9-11] Although etiological factors related to ECC in young children have been well studied, the impact of each factor may differ in each population due to cultural, genetic, and socioeconomic factors.

This study's aim was to investigate the salivary counts of *Streptococcus mutans*, lactobacilli, and *Candida albicans* and the prevalence of early childhood caries in 15- to 35-month-old Turkish children and the associations with the mothers' characteristics such as caries experience, education levels, socioeconomic criteria, and children's feeding habits.

METHODS

STUDY POPULATION

The study population consisted of children receiving routine medical screenings in the Well Child Care Outpatient Clinic at the Faculty of Medicine, Ege University, Bornova-Izmir, Turkey. Only children whose mothers consented participated in this study. This outpatient clinic is located in an urban area and provides health care to children insured by the government. There were 101 children (53 boys and 48 girls) 15 to 35 months old who participated in this study with their mothers. The mean ages of the children and their mothers were 24.6 (±4.3) months and 32.8 (±5.8) years, respectively. All participants were local residents, not immigrants.

The study protocol was approved by the Ethical Committee of Ege University, and informed consent was obtained from all mothers. Children who had taken antibiotic therapy within 3 months before the dental examination and children with any systemic disease were excluded.

QUESTIONNAIRE

The mothers were interviewed by 2 of the authors based on a prepared questionnaire, as shown in Figure 1.

DENTAL EXAMINATION

The children and their mothers were examined by the same authors under daylight conditions in a waiting room. The examiners were trained for caries identification and to evaluate plaque on dental patients who were not part of the study for 2 weeks. Inter- and intrareproducibility was calculated using Cohen-Kappa scores and recorded as 0.86 and 0.92, respectively. The mothers' caries status was recorded according to World Health Organization criteria.[12] For ECC diagnosis, the National Institute of Dental and Craniofacial Research's (NIDCR) definition was used, which was based on the presence of 1 or more primary teeth affected.[13] White spot decalcifications were not considered to be caries, and loss of mineral substance with cavitation was recorded as caries.

Before dental examination of the children, wet sterile gauze pads were used to clean all teeth surfaces. The dental examination was performed using a visual and nontactile technique ("lift the lip") without probing.

Plaque scores of mother-child pairs were evaluated using the criteria of Silness and Löe[14]: 0=no plaque;

1= a film of plaque adhering to the tooth's free gingival margin and adjacent area;
2= moderate accumulation of soft deposits within the gingival pocket, or the tooth and gingival margin which can be seen with the naked eye;
3= abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

The plaque indices were calculated by summing the scores for all teeth surfaces and dividing by the number of surfaces.

BACTERIAL EXAMINATION

Whole nonstimulated saliva samples were obtained from each child for isolation of *S mutans,* lactobacilli, and *C albicans*. These samples were obtained by saturating cotton pellets in saliva that pooled in the floor of the mouth. The pellets were immediately placed in vials containing 1 ml of phosphate-buffered saline and placed on ice. The saliva samples were transported to the laboratory and processed within 2 hours. They were sonicated for 30 seconds to disperse bacteria from cotton pellets and serially diluted in phosphate-buffered saline. From each of the dilutions, 0.1-ml samples were plated on Mitis-Salivarius (MS) agar (Difco, Detroit, Mich) with 15% sucrose (Difco, Detroit, Mich) and 0.2 units/ml of bacitracin (Sigma, Sigma-Aldrich Co, St. Louis, Mo), prepared according to Gold et al.[15] The agar plates were then incubated at 37°C in a 5% CO_2 atmosphere for 48 hours. The numbers of *S mutans* were identified by colony morphology using a light microscope. *S mutans* strain NCTC 10449 was also incubated as a control plate to confirm bacterial identification. Saliva samples were also plated on Rogosa agar[16] and Albicans ID agar (Biomeriux SA, Marcy-I'etoile, France)[17] for

Child's name/surname:: _____ Date _____

Phone number: _____

Gender: _____

Birth date (month): _____

Birth weight (g): _____

Gestation period (week): _____

Primary caregiver of the children: a) mother _____ b) caretaker _____ c) grandmother _____ d) kindergarten _____

Maternal age: a) mother _____

Maternal education level:

Mother: a) Primary level (5 years) _____ b) <Secondary level (6-11 years) _____ c) >Secondary level (>11 years) _____

Family income (per month): _____

No. of meals of the child: _____

Duration of breast-feeding: a) 1-12 months _____ b) >12 months _____

Maternal sharing of utensils: a) Yes _____ b) No _____

Tooth-brushing habits: a) 1/day _____ b) 2/day _____ c) Irregular _____ d) None _____

Using pacifier: a) Use _____ b) Not use _____

First primary tooth eruption: a) 0-6 months b) ≥7 months

dfs/DMFS scores (WHO): Child: _____ Mother: _____

Plaque index scores (Silness and Löe, 1964): Child: _____ Mother: _____

Microbiological counts: *Streptococcus mutans* _____ Lactobacilli _____ *Candida albicans* _____

Figure 1. The questionnaire carried out to mothers.

the estimation of lactobacilli and *C albicans*, respectively.

Fine opaque colonies on Rogosa agar that were gram positive, catalase negative, rod-shaped cells were considered to be lactobacilli species. Smooth blue colonies were identified as *C albicans*, and other type of colonies were considered to be non-albicans yeasts. The germ-tube test and carbohydrate fermentation tests were also used for identification. The levels of *S mutans* and lactobacilli were interpreted according to the criteria described by Roeters et al.[18]

All data were analyzed with the SPSS version 10.0 software program (SPSS Inc, Chicago, Ill). The majority of data were not normally distributed; therefore, nonparametric methods, including Mann-Whitney and Spearman rank correlation tests, were used for the comparisons and correlations between groups. Chi-square or Fisher exact tests were used to analyze the categorical variables. Logistic regression models were built up to assess and estimate the odds ratios of caries experience and background variables. The statistical level of significance was set at $P<.05$.

RESULTS

The colonization of salivary *S mutans* was found in 49% of the children (N=50), with a mean (±SD) of $7.1x10^3$ ($±2.7x10^4$). Lactobacilli were detected in all of the children, with a mean of $2.4x10^5$ ($±4.7x10^5$). *C albicans* was found in 9% (N=9) of the children with a mean of $9.3x10^0$ ($±6.1x10^2$). ECC prevalence was 9% (N=9) in the study group. The mean number of carious teeth per child was 0.6 (±2.9). The mean DMFS score of the mothers was 9.7 (±8.7). In the logistic analysis, the mothers' DMFS scores were found to be an impact factor on the children's caries experience (odds ratio=1.074; 95% confidence interval=1.0-1.15). The mean plaque scores were 1.2 (±0.6) and 1.9 (±0.6) in the children and their mothers, respectively. The correlation between ECC and plaque scores was found to be statistically significant (P=.029). Children having a plaque score of 1 had a dfs level of 0.1 (±0.6), and children with plaque scores of 2 and 3 had dfs levels of 1.1 (±3.4) and 3.8 (±4.2), respectively. There was also a positive correlation between ECC and the levels of *S mutans* counts (P=.034), which is demonstrated in Table 1. There was no significant correlation between plaque scores and salivary *S mutans* and lactobacilli counts (P=.279 and P=.112, respectively).

In relation to feeding habits, the mean duration of breast-feeding was 12.1 (±6.9) months, ranging from 1 to 26 months. Half of the children (N=48) were breast-fed more than 1 year, while 6 of them were still breast-fed at the time of the interview. The mean breast-feeding frequency was 4 times per day. The relationship between the duration of breast-feeding and *S mutans* counts was not statistically significant. A positive, but not statistically significant, correlation, however, was found between lactobacilli and *C albicans* counts and the duration of breast-feeding. Twenty-nine percent (N=29) of the children had used a pacifier, with a mean duration of 4.9 (±8.1) months, ranging from 2 to 30 months. A positive correlation was observed between the duration of using pacifier and lactobacilli counts (P=.020).

Forty-five children (45%) were using a feeding bottle containing sweetened milk for more than a year. Of these children, 26 (58%) were using a feeding bottle during sleep. The relationship between prolonged usage of feeding bottle

(>12 months) with sweetened milk and *S mutans* counts was statistically significant (P=.046). The mean number of meals per day was 4. Almost half of the children (N=49) were fed by infant formula, with a mean initial time of 4.2 (±2.6) months. In the sample group, the mean age of eruption of the lower primary central incisors was 7.4 (±1.9) months. The associations between the salivary microbiological counts and ECC and etiological factors were presented in Table 2.

The principal caregivers were mothers in 47% (N=47) and caretakers in 23% (N=23) of the children. The percentage of children who were cared for by their grandmothers was 27% (N=27), and 4 of them (4%) were attending a kindergarten. No correlation was found between the principal caregiver and microbiological counts of the children and ECC.

The results of mothers sharing utensils and food with children were quite high in the study group. Thirty-four (34%) of the mothers reported that they put the child's spoon into their mouth during feeding. While a significant correlation between maternal sharing and *C albicans* counts was found (P=.037), no correlation was found for *S mutans* and lactobacilli (P>.05).

More than half of the mothers (N=53) had graduated from high school (>11 years), while 23 graduated from primary school (5 years) and the others (N=25) had an education of 5 to 11 years. A negative correlation was observed between maternal education and the children's *S mutans* counts (P=.026). More than half of the families had a family income of $750 per month, which is a medium family income in Turkey. Twenty-seven percent (N=27) of

Table 1. The Mean Scores of dfs Distributed According to Children's Streptococcus mutans and Lactobacilli Levels

	S mutans			Lactobacilli		
	$0<x<10^4$ Low	$10^4<x<10^6$ Moderate	$x\geq10^6$ High	$0<x<10^3$ Low	$10^3<x<10^5$ Moderate	$x\geq10^5$ High
Detected	41 (40%)	9 (9%)	–	8 (8%)	46 (45%)	47 (46%)
dfs (mean±SD*)	0.26±1.02	4.66±8.7†	–	0.25±0.70	0.53±3.21	0.76±2.86

***Standard deviation.**
***Statistically significant.**

Table 2. The Mean Scores of dfs and Microbiological Counts in Relation to Etiological Factors

		dfs	*S mutans*		Lactobacilli			*C albicans*	
			detected	not detected	low	moderate	high	detected	not detected
	n	Mean (±SD*)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Maternal education									
Primary level (5 ys)	23	0.98 (±3.91)	14 (28)	9 (18)	4 (50)	7 (15)	12 (26)	3 (33)	20 (22)
<secondary level (6-11 ys)	25	0.26 (±1.05)	13 (26)	12 (24)	3 (38)	10 (22)	12 (26)	4 (44)	21 (23)
>secondary level (>11 ys)	53	0.16 (±0.80)	23 (46)	30 (58)†	1 (12)	29 (63)	23 (48)	2 (23)	51 (55)
Breast-feeding duration									
1-12 mos	56	0.30 (±1.12)	17 (34)	39 (76)	3 (38)	24 (52)	29 (61)	4 (44)	52 (57)
>12 mos	45	0.40 (±1.89)	33 (66)†	12 (24)	5 (62)	22 (48)	18 (39)	5 (56)	40 (43)
Maternal sharing of utensils									
Yes	34	1.47 (±4.8)	15 (30)	19 (37)	1 (13)	16 (35)	17 (36)	6 (67)†	28 (30)
No	67	0.17 (±0.81)	35 (70)	32 (63)	7 (87)	30 (65)	30 (64)	3 (33)	64 (70)
Child's caregiver									
Mother	47	0.70 (±3.28)	20 (40)	27 (53)	6 (75)	20 (43)	21 (45)	3 (33)	44 (48)
Caretaker	23	0.08 (±0.28)	13 (26)	10 (20)	1 (12.5)	9 (20)	13 (28)	2 (22)	21 (23)
Kindergarten	4	0.00 (±0.00)	2 (4)	2 (3)	0 (0.0)	3 (7)	1 (1)	0 (0)	4 (4)
First primary teeth eruption									
0-6 mos	45	1.05 (±3.86)	25 (50)	20 (39)	3 (38)	17 (37)	25 (53)	4 (44)	41 (45)
≥7 mos	56	0.10 (±0.33)	25 (50)	31 (61)	5 (62)	29 (63)	22 (47)	5 (56)	51 (55)
Using a pacifier									
Not use	45	1.05 (±3.86)	25 (50)	20 (39)	3 (38)	17 (37)	25 (53)	4 (44)	41 (45)
Use	29	1.23 (±3.96)	18 (36)	11 (22)	2 (25)	7 (15)	20 (43)†	6 (67)	23 (25)

***Standard deviation.**
***Statistically significant.**
***Feeding bottle containing sweetened milk.**

the families had a high (>$750) and 21% (N=21) of the families had a low family income (≤$400 per month). No statistically significant relationship was found between salivary microbiological counts, ECC, and family income.

The percentage of the mothers who brushed their children's teeth regularly with fluoridated toothpaste was 50%. The relationship between tooth-brushing and ECC and salivary microbiological counts was not found to be statistically significant.

The mean birth weight of the children was 3.3 (0.5) kg, with 92 children (91%) at normal birth weight and 9 children (9%) at preterm low birth weight. Fifteen mothers (15%) had systemic disease (diabetes mellitus, hypertension) during pregnancy. No significant relationship existed between the low birth weight and mother's disease during pregnancy and microbiological counts of the children and ECC.

DISCUSSION

In the present study, *S mutans* was detected in the salivary counts of 49% of the children (N=50). The salivary counts of *S mutans* showed similarity in some studies,[10,18,19] whereas it was reported higher or lower at the same age group.[20,21] The variation in the results could be explained by differences in early colonization of *S mutans*, feeding habits, and the microbiological methods used. Thus, almost half of the children (45%) were using a feeding bottle with sweetened milk for more than a year, and this was significantly correlated with salivary *S mutans* counts. These results confirmed the study of Grindefjord et al,[22] in which a correlation was found between the consumption of sugar-containing beverages and the presence of *S mutans* in infants. The present authors suggested that the early colonization of *S mutans* in the study population, combined with prolonged bottle-feeding, could be predictive of the child's caries risk. This study's results were also in accordance with Tinanoff et al,[23] who indicated both early infection of cariogenic bacteria and frequent exposure to a cariogenic diet as the basic reasons for tooth demineralization in very small children. Furthermore, a positive correlation between ECC and children's salivary *S mutans* levels was demonstrated, supporting the results of some investigators who suggested that children with caries have higher *S mutans* counts than caries-free children.[24-26]

In the present study, the prevalence of ECC was 9% using the NIDCR's definition, which classified a child with 1 or more affected teeth as having ECC. Most of the children in the present study were diagnosed as caries free (91%). This finding was similar to the results of Weerheijm et al[6] and to the age of their sample group. The prevalence of ECC, however, varies from 2% to as high as 90% due to the inconsistent case definitions and diagnostic criteria for caries and localization which make comparisons among studies difficult. [27-29] In the present study, the low prevalence of ECC may be related to the selected area (urban), diagnostic criteria, and the children's younger age. This is also mentioned by some authors who found age to be the most important predictor of caries experience and that the caries approximately doubled for every additional year of age.[25,30]

It is reported that low socioeconomic status, ethnic minority, and minimal parental education may have an impact on the distribution of ECC. The mothers' oral health factors—such as high MS levels, high caries experience, and poor oral hygiene—could be important risk indicators for caries for their children.[9,26,31-33] In this study, the logistic analysis indicated that the mothers' DMFS scores were an impact factor for caries in their children, which was in accordance with the results of the recent studies.[9-11] In the authors' previous study, it was found that the mothers who harbored high levels of *S mutans* with high DMFS scores shared the similar genotypes of *S mutans* with their 2- to 3-year-old children.[34] Thus, mothers with high *S mutans* levels and high DMFS scores may affect the child's early infection with *S mutans*, resulting in an increase of ECC. Furthermore, the *S mutans* counts in children were correlated inversely with the mothers' education levels. Thus, children whose mothers had low education levels were more likely to have early colonization of *S mutans*.

Because breast milk is nutritionally rich, breast-feeding is recommended in Turkish children until 24 months of age. In this study, the mean duration of breast-feeding was 12 months (±6.8) and more than half the children had been breast-fed more than a year. Similar to some previous studies, however, the relationship between the duration of breast-feeding and *S mutans* counts and ECC was not found statistically significant.[6,20] Some authors, however, suggested that prolonged breast-feeding led to higher caries prevalence and allowed the colonization and proliferation of MS and lactobacilli on the teeth of young children.[22,35-37]

It is reported that the amount of salivary MS has been also significantly correlated with oral hygiene in children.[24] In the present study, the association between ECC and the children's plaque scores was determined to be statistically significant, but no association could be found between the amounts of salivary microbiological counts and plaque in children. The study also failed to show a correlation between tooth-brushing and salivary microbiological counts and caries. It is suggested that the mothers overestimated the frequency of brushing in their children.

The frequency of salivary lactobacilli and *C albicans* found during the first years of life varied in the studies. Ollila et al[38] found the prevalence of lactobacilli to be 18% at 2½ years old, and Grindefjord et al[22] found a 22% prevalence at the same age. Salivary candida has been found in 24% to 30% of the children between 3 and 12 years of age in the studies of Ollila et al[38] and Weerheijm et al,[6] respectively. In the present study, the prevalence of lactobacilli and *C albicans* was 100% and 9%, respectively. Furthermore, a statistically significant correlation was found between the duration of using pacifier and lactobacilli counts and maternal sharing and *C albicans.* The variation of the results could be explained by differences in lactobacilli and *C albicans* colonization and feeding habits.

One of this study's limitations is the possibility that some children have been classified as caries free, although they have white spot lesions with no cavitation. Other false negatives could have resulted if the amount of obtained salivary samples were not enough for cultivation in some children. Moreover, inaccurate replies to the questionnaire were undetectable and unavoidable and would be expected to produce false negatives rather than false positives. Validation of the questionnaire is an objective in further studies. Another limitation of this study was that it is not known how the mothers' risk indicators or the children's caries status might have changed over time. Some of the children who were caries free might be destined to develop caries in the future. An enlarged study group and, ideally, a longitudinal study is needed to better evaluate small effect sizes and interaction effects, confirm the authors' results, and evaluate maternal risk indicators as predictors of childhood caries.

CONCLUSIONS

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

1. Maternal factors—such as high DMFS scores, low education levels, prolonged bottle-feeding with sweetened milk, pacifier use, and maternal sharing—were strong risk indicators for identifying high caries-susceptible children.
2. Considering the maternal risk indicators, antibacterial measures aimed at reducing the number of caries-related microorganisms in children should also be applied to mothers who have high DMFS scores in order to prevent caries progression in their children.
3. Further longer-term studies with an enlarged study group are needed to confirm the authors' results in evaluating the risk indicators as predictors of childhood caries.

REFERENCES

1. Milnes AR. Description and epidemiology of nursing caries. J Public Health Dent 1996;56:38-50.
2. Berkowitz RJ, Jones P. Mouth-to-mouth transmission of the bacterium Streptococcus mutans between mother and child. Arch Oral Biol 1983;30:377-379.
3. Alaluusua S. Transmission of mutans streptococci. Proc Finn Dent Soc 1991;87:443-447.
4. Caufield PW, Cutter GR, Dasanayake AP. Initial acquisition of mutans streptococci by infants: Evidence for a discrete window of infectivity. J Dent Res 1993;72:37-45.
5. Eronat N, Eden E. A comparative study of some influencing factors of rampant or nursing caries in preschool children. J Clin Pediatr Dent 1992;16:275-279.
6. Weerheijm KL, Uyttendaele-Speybrouch BFM, Euwe HC, Groen HJ. Prolonged demand breast-feeding and nursing caries. Caries Res 1998;32:46-50.
7. Schou L, Vitenbroek D. Social and behavioral indicators of caries experience in 5-year-old children. Community Dent Oral Epidemiol 1995;23:276-281.
8. Stecksen-Blicks L, Holm AK. Between meal eating, tooth-brushing frequency, and dental caries in the north of Sweden. Int J Paediatr Dent 1995;5:67-72.
9. Smith RE, Badner VM, Morse DE, Freeman K. Maternal risk indicators for childhood caries in an inner city population. Community Dent Oral Epidemiol 2002;30:176-181.
10. Wan AKL, Seow WK, Purdie DM, Bird PS, Walsh LJ, Tudehope DI. Oral colonization of Steptococcus mutans in six-month-old predentate infants. J Dent Res 2001;80:2060-2065.
11. Vachirarojpisan T, Shinada K, Kawaguchi Y, Laungwechakan P, Somtoke T, Detsomboonrat P. Early childhood caries in children aged 6-19 months. Community Dent Oral Epidemiol 2004;32:133-142.
12. World Health Organization. *Oral Health Surveys: Basic Methods*. 4th ed. Geneva Switzerland: WHO; 1997.
13. Drury TF, Horowitz AM, Ismail AI, Maertens MP, Rozier RG, Selwitz RH. Diagnosing and reporting early childhood caries for research purposes. J Public Health Dent 1999;59:192-197.
14. Silness J, Löe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964;22:121-135.
15. Gold OG, Jordan HV, Van Houte JA. Selective medium for Streptococcus mutans. Arch Oral Biol 1973;18:1357-1364.
16. Rogosa, M, Mitchell JA, Wiseman RF. A selective medium for the isolation of oral and faecal lactobacilli. J Bacteriol 1951;62:132-133.
17. Cardenes CD, Carrillo AJ, Arias A, et al. Comparison of albicans ID2 agar plate with the germ tube for presumptive identification of Candida albicans. Diagn Microbiol Infect Dis 2002;42:181-185.
18. Roeters FJM, van de Hoeven JS, Burgersdijk RCW, Schaeden MJM. Lactobacilli, mutans streptococci, and dental caries: A longitudinal study in 2-year-old children up to the age of 5 years. Caries Res 1995;29:272-279.
19. Fujiwara T, Sasada E, Mima N, Ooshima T. Caries prevalence and salivary mutans streptococci in 0- to 2-year-old children of Japan. Community Dent Oral Epidemiol 1991;19:151-154.
20. Thibodeau EA, O'Sullivan DM, Tinanoff N. Mutans streptococci and caries prevalence in preschool children. Community Dent Oral Epidemiol 1993;21:288-291.
21. Radford JR, Ballantyne HM, Nugent Z, Beighton D, Robertson M, Longbottom C. Caries-associated microorganisms in infants from different socioeconomic backgrounds in Scotland. J Dent 2000;28:307-312.
22. Grindefjord M, Dahllöf G, Wikner S, Höjer B, Modeer. Prevalence of mutans streptococci in 1-year-old children. Oral Microbiol Immunol 1991;6:280-283.

23. Tinanoff N, Kaste LM, Corbin SB. Early childhood caries: A positive beginning. Community Dent Oral Epidemiol 1998;26:117-119.
24. Habibian M, Beighton D, Stevenson R, Lawson M, Roberts G. Relationship between dietary behaviors, oral hygiene, and mutans streptococci in dental plaque of a group of infants in southern England. Arch Oral Biol 2002;47:491-498.
25. Milgrom P, Riedy CA, Weinstein P, Tanner ACR, Manibusan L, Bruss J. Dental caries and its relationship to bacterial infection, hypoplasia, diet, and oral hygiene in 6- to 36-month-old children. Community Dent Oral Epidemiol 2000;28:295-306.
26. Thorild I, Lindau-Jonson B, Twetman S. Prevalence of salivary Streptococcus mutans in mothers and in their preschool children. Int J Paediatr Dent 2002;12:2-7.
27. Horowitz HS. Research issues in early childhood caries. Community Dent Oral Epidemiol 1998;26:67-81.
28. Grauwe A, Aps, JKM, Martens JKM. Early childhood caries (ECC): What's in a name? Eur J Paediatr Dent 2004;2:62-70.
29. Ismail AI, Sohn W. A systematic review of clinical diagnostic criteria of early childhood caries. J Public Health Dent 1999;59:171-191.
30. Gibson S, Williams S. Dental caries in preschool children: Associations with social class, tooth-brushing habit, and consumption of sugars and sugar-containing foods. Caries Res 1994;33:101-113.
31. King JM, Pitter AFV, Edwards H. Some social predictors of caries experience. Br Dent J 1983;155:266-268.
32. Milen A. Role of social class in caries occurrence in primary teeth. Int J Epidemiol 1987;16:252-256.
33. Ramos-Gomez FJ, Weintraub JA, Gansky SA, Hoover CI, Featherstone JD. Bacterial, behavioral, and environmental factors associated with early childhood caries. J Clin Pediatr Dent 2002;26:165-173.
34. Ersin NK, Kocabas EH, Alpoz AR, Uzel A. Transmission of Streptococcus mutans in a group of Turkish families. Oral Microbiol Immunol 2004;19:408-410.
35. Ripa LW. Nursing caries: A comprehensive review. Pediatr Dent 1988;10:268-282.
36. Li Y, Wang W, Caufield PW. The fidelity of mutans streptococci transmission and caries status correlate with breast-feeding experience among Chinese families. Caries Res 2000;34:123-132.
37. Matee MI, Mikx FH, Maselle SY. Mutans streptococci and lactobacilli in breast-fed children with rampant caries. Caries Res 1992;26:183-187.
38. Ollila P, Niemela M, Uhari M. Risk factors for colonization of salivary lactobacilli and candida in children. Acta Odontol Scand 1997;55:9-13.