A long-term effect of caries-related factors in initially caries-free children

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Aim. The aim of this study was to examine the rate and pattern of early childhood caries development in caries-free children based on their dental plaque accumulations, salivary *Streptococcus mutans* levels, maternal sharing, oral hygiene, and feeding attitudes at baseline and at 24-month follow-up period.

Design. A total of 92 children, aged between 15 to 35 months, comprised the study group. The children's dental examinations were first carried out at baseline, and 56 of them were re-examined 24 months later to determine the changes in dental status. The

mothers were also interviewed at each examination based on a prepared questionnaire.

Results. The prevalence of caries incidence was 45% at 24-month evaluation period among initially caries-free children. The new caries formation was mostly observed on occlusal and aproximal surfaces of maxillary molars (28% and 26%) followed by mesial, distal and buccal surfaces of anterior teeth, respectively. Significant correlations between dental caries formation and maternal sharing, *S. mutans* levels, and plaque scores were found.

Conclusion. The results indicated that early *S. mutans* colonization, high plaque accumulation, and maternal sharing were important factors on a child's caries development.

Introduction

Dental caries is a multifactorial disease that is particularly related to the presence of cariogenic microorganisms, fermentable carbohydrates in the diet, susceptible tooth and host, and the time^{1,2}. Some factors such as socioeconomic status, parental education level and the maternal flora are also essential for the initiation and the progression of the disease^{3,4}.

Early childhood may be the most important period for the future dental health. During this period, the primary teeth erupt, bacteria colonize on the teeth, and dental attitudes are gained⁵. A precursor to the development of dental caries is the accumulation of bacterial dental plaque^{5,6}.

Dental caries can also be characterized as a sugar-induced infectious bacterial disease in which the child's primary dentition is severely

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damaged⁷. It has been shown that the use of feeding bottles containing sugar is an important predisposing factor in the prevalence of caries⁸. Prolonged breastfeeding may also be important in some communities^{8,9}.

Mutans streptococci (MS), particularly *Streptococcus mutans*, are also considered to be the aetiologic agents of dental caries¹⁰. It has been reported that children whose teeth are colonized by MS early in life show a higher caries experience than those colonized later or not at all^{4,10,11}. Infants most likely become colonized from their parents or other individuals with whom they have close contact, and infants whose mothers harbour higher levels of MS in saliva become colonized more readily than infants of mothers with low salivary MS levels^{9,11-14}.

Since there are few longitudinal data on caries risk factors in children, the aim of this study was to examine the rate and pattern of early childhood caries (ECC) development in caries-free Turkish children based on their plaque accumulations, salivary *S. mutans* levels, oral hygiene, and feeding attitudes at baseline and at 24-month follow-up period.

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Materials and 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, Izmir, Turkey. A total of 92 children (50 boys and 42 girls), aged between 15 to 35 months (with the mean age 24.3 ± 4.1 months), comprised the study group. The children's dental examinations were first carried out at baseline, and 56 of them were re-examined at 24 months to determine the changes in dental status over time.

The study protocol was approved by the Ethics Committee of Ege University and written informed consent was obtained from all mothers. Children who had taken antibiotic therapy within 3 months prior to the baseline and final dental examinations and children with any systemic disease were not included to the study.

Baseline dental examination

The mothers were interviewed by two of the authors (D.C. and N.K.E.) based on a prepared questionnaire that includes questions on child's age and gender, oral hygiene, feeding attitudes, duration of breastfeeding, primary caregiver mother's age, maternal education level, and maternal sharing of utensils.

The children were examined by the same authors under daylight conditions. The examiners were trained for caries identification and to evaluate plaque for 2 weeks. Inter- and intrareproducibility was calculated using Cohen-Kappa scores and recorded as 0.86 and 0.92, respectively.

Plaque scores were assessed by Silness and Löe plaque index¹⁵. The caries status of the children was recorded according to World Health Organization criteria¹⁶. The description of ECC (as ECC and severe ECC) was performed by Drury *et al.*¹⁷. 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 non-tactile technique ('lift the lip') without probing.

Final dental examination

Two years after baseline examination, 56 cariesfree children were re-evaluated and their mothers were interviewed by the same authors based on a new questionnaire that includes the same questions about mother–child pairs. Dental examinations of children were performed again as described before.

Baseline bacterial examination

Whole unstimulated saliva samples were obtained from each child for the isolation of S. mutans. These samples were obtained by saturating cotton pellets in saliva that pooled in the floor of the mouth. The amount of saliva sample was determined by using microweight (Sartorius Expert LE225D-0CE, Göttingen, Germany). 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 h. They were sonicated for 30 s to disperse bacteria from cotton pellets and serially diluted in phosphatebuffered saline. From each of the dilutions, 0.1-mL samples were plated on mitis salivarius agar (Difco, Detroit, MI, USA) with 15% sucrose (Difco) and 0.2 units/mL of bacitracin (Sigma, Sigma-Aldrich Co., St Louis, MO, USA), prepared according to Gold *et al.*¹⁸. The agar plates were then incubated at 37 °C in 5% CO₂ atmosphere for 48 h. 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. The level of S. mutans was interpreted according to the criteria described by Roeters *et al.*¹⁹.

Final bacterial examination

Whole unstimulated saliva samples were obtained from each child for isolation of *S. mutans* at the 24-month recall visit as described before. *S. mutans* colonies were recorded as colony-forming units per millilitre.

Statistical analysis

All data were analysed with the SPSS version 11.0 software program (SPSS Inc., Chicago, IL,

USA). Mann–Whitney and Spearman rank correlation tests were used for the comparisons and correlations between the groups. Chi-squared or Fisher exact tests were used to analyse the categorical variables. The statistical level of significance was set at P < 0.05. Logistic regression models were built up to assess and estimate the odd ratios of caries experience and background variables.

Results

The mean ages (\pm SD) of the 56 children (26 male, 30 female) and their mothers at the final examination were 4.29 ± 0.56 and 35.88 ± 5.57 years, respectively. A total of 55% (n = 31) of the children were still caries-free at the final examination. The mean (\pm SD) final dft and dfs scores were 1.00 ± 1.53 and 1.18 ± 1.96 , respectively. No significant differences were found between age, gender, and final dft and dfs scores. The new caries formation was mostly observed on occlusal and aproximal surfaces of maxillary molars (28% and 26%) followed by mesial, distal, and buccal surfaces of anterior teeth, respectively.

Regarding the mother's education, about half of them (n = 27, 48%) had high education (> 11 years). No significant correlation was found between final dft and dfs scores and mothers' education levels and age.

About half of the children (n = 26, 46%)were breast-fed more than 1 year at final examination. The correlation between final dft and dfs scores and breastfeeding was not statistically significant. At baseline, 45 children (49% of 92 children) were using feeding bottles containing sweetened milk for more than a year, and of these children, 26 (58% of 45 children) were using feeding bottles during sleep. At the final examination, it was detected that 13 of these children were still using feeding bottles. No significant correlation was found between prolonged usage of feeding bottle and final dft and dfs scores. The correlation between feeding bottle during sleep and S. mutans counts (baseline and final counts) was not statistically significant.

It was recorded that only 50 (54%) of the mothers brushed their child's teeth regularly twice a day at baseline, while 31 (55%) of them

brushed at the final examination. The correlation between final dft and dfs scores and tooth brushing attitude at baseline and the final examinations was not found statistically significant.

The principal caregivers were mothers in 47% (n = 26) and caretakers in 9% (n = 5) of the children. The percentage of children who were cared for by their grandmothers was 14% (n = 8), and 30% of them (n = 17) were attending a kindergarten. No correlation was found between the principal caregiver at baseline and final dft and dfs scores.

Thirty-four (37%) of the mothers reported that they put the child's spoon into their mouth during feeding at baseline while the ratio was decreased to 16% (n = 9) at the final examination. A significant correlation was found between initial maternal sharing and final dft and dfs scores (P = 0.043 and 0.037, respectively).

The colonization of salivary *S. mutans* was found in 49 (91%) of the children at final examination, with a mean (\pm SD) level of 6.3×10^4 ($\pm 2.4 \times 10^3$) CFU/mL. Among these 49 infected children, 13 of them were *S. mutans* free at baseline. The distribution of mean final dft and dfs scores according to the salivary *S. mutans* levels are shown in Table 1. A positive significant correlation was detected between the baseline *S. mutans* levels and final dft and dfs scores (P = 0.004 and 0.005). The correlation between the final *S. mutans* levels and final dft and final dft and dfs scores was also statistically significant (P = 0.003 and 0.004).

The distribution of mean final dft and dfs scores according to the presence of dental plaque at baseline and final examination are shown in Table 2. A positive significant correlation was found between dental plaque scores at baseline and final dft and dfs scores (P = 0.001 and 0.001). The correlation between final plaque scores and final dft and dfs scores was also statistically significant (P = 0.003 and 0.002).

All caries-related factors analysed in the present study are shown in Table 3.

In the logistic analysis, the baseline and final salivary *S. mutans* levels, dental plaque scores, and initial maternal sharing were found to be important factors on a child's caries development (Table 4).

	Salivary <i>S. mutans</i> levels at baseline (CFU/mL)			Salivary <i>S. mutans</i> levels at the final examination (CFU/mL)		
	Low	Moderate	High	Low	Moderate	High
	0 < <i>x</i> ≤ 10 ⁴	10⁴ < <i>x</i> ≤ 10 ⁶	<i>x</i> > 10⁵	0 < <i>x</i> ≤ 10 ⁴	10⁴ < <i>x</i> ≤ 10 ⁶	<i>x</i> > 10 ⁶
Final dft scores mean (± SD)	0.63 (± 0.80)*	0.78 (± 1.67)	1.92 (± 1.65)*	0.83 (± 1.55)‡	1.29 (± 1.63)	1.40 (± 1.34)‡
Final dfs scores mean (± SD)	0.75 (± 1.12)†	0.85 (± 1.97)	2.38 (± 2.36)†	1.00 (± 2.09)§	1.43 (± 1.78)	1.80 (± 1.78)§

Table 1. Distribution of mean art and all scores according to the sanvary streptococcus matans level	Table 1.	Distribution	of mean	dft and	dfs scores	according	to the	salivary	Streptococcus	mutans	level
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*Final dft scores were statistically significant between *S. mutans* low and high groups at baseline (P = 0.004). †Final dfs scores were statistically significant between *S. mutans* low and high groups at baseline (P = 0.005). ‡Final dft scores were statistically significant between *S. mutans* low and high groups at final examination (P = 0.003). §Final dfs scores were statistically significant between *S. mutans* low and high groups at final examination (P = 0.003). §Final dfs scores were statistically significant between *S. mutans* low and high groups at final examination (P = 0.004). SD, standard deviation.

Table 2. Distribution of mean dft and dfs scores according to the dental plaque accumulation.

	The presence o	The presence of dental plaque		The presence of dental plaque		
	at ba	at baseline		at the final examination		
	Present	Not present	Present	Not present		
Final dft scores mean (± SD)	1.59 (± 1.65)*	0.62 (± 1.34)*	1.38 (± 1.72)‡	0.26 (± 0.65)‡		
	(<i>n</i> = 22)	(<i>n</i> = 34)	(<i>n</i> = 37)	(<i>n</i> = 19)		
Final dfs scores mean (± SD)	1.86 (± 2.10)†	0.74 (± 1.76)†	1.65 (± 2.23)§	0.26 (± 0.65)§		
	(n = 22)	(<i>n</i> = 34)	(n = 37)	(n = 19)		

*Final dft scores were statistically significant between *Streptococcus mutans* low and high groups at baseline (P = 0.001). †Final dfs scores were statistically significant between *S. mutans* low and high groups at baseline (P = 0.001). ‡Final dft scores were statistically significant between *S. mutans* low and high groups at final examination (P = 0.003). §Final dfs scores were statistically significant between *S. mutans* low and high groups at final examination (P = 0.003). §Final dfs scores were statistically significant between *S. mutans* low and high groups at final examination (P = 0.002). SD, standard deviation.

Caries-related factors	At baseline examination $(n = 92)$	At final examination $(n = 56)$		
Bottle feeding during sleep	28% (<i>n</i> = 26)	23% (<i>n</i> = 13)		
Maternal sharing of utensils	37% (<i>n</i> = 34)	16% (<i>n</i> = 9)		
Oral hygiene attitude	 Twice a day regularly: 54% (n = 50) Irregular: 46% (n = 42) 	 Twice a day regularly: 55% (n = 31) Irregular: 45% (n = 25) 		
Streptococcus mutans levels	 Not detected (n = 47) 0 < x = 10⁴ (n = 39) 10⁴ < x = 10 (n = 6) x > 10⁶ (n = 0) 	• Not detected $(n = 7)$ • $0 < x = 10^4$ $(n = 34)$ • $10^4 < x = 10^6$ $(n = 14)$ • $x > 10^6$ $(n = 1)$		
Visible dental plaque	 Present 56% (n = 52) Not present 44% (n = 40) 	 Present 66% (n = 37) Not present 34% (n = 19) 		
Dental caries score	 Caries free 91% (n = 84) ECC (n = 1) 6% (n = 6) Severe ECC (> 1) 3% (n = 2) 	 Caries free 55% (n = 31) ECC (n = 1) 17% (n = 10) Severe ECC (> 1) 28% (n = 15) 		

ECC, early childhood caries.

Discussion

In this 2-year follow-up study, caries-related factors regarding the feeding and oral hygiene attitudes, maternal sharing, plaque accumulations as well as microbiological factors were investigated since there are limited longitudinal data in the literature^{20,21}. It was found that 25 (45%) out of 56 initially caries-free children had developed caries at the 24-month examination. The main difficulty with longitudinal studies is that some participants are not followed throughout the study since they move or lose interest. Only 61% (56/92) of the

Variables		Р	OR	95% CI
Baseline Streptococcus mutans level	Low (ref.)	0.010	11.927	1.750–81.294
	Moderate-high			
Final Streptococcus mutans level	Low (ref.)	0.011	11.736	1.151–83.034
	Moderate-high			
Baseline dental plaque	Present	0.010	7.154	1.601-31.965
	Not present (ref.)			
Final dental plaque	Present	0.017	7.880	1.635–37.968
	Not present (ref.)			
Initially maternal sharing	Yes	0.045	7.414	0.967-56.860
	No (ref.)			

Table 4. Logistic regression: *P*-values, odd ratios and 95% confidence internals for the association between dental caries and risk factors.

Forward stepwise (likelihood ratio) $\chi^2 = 31.299$, P < 0.001.

participants followed up throughout the present study.

The formation of new caries was mostly observed on occlusal and aproximal surfaces of maxillary molars. This is in contrast to many other studies^{22,23}. The high rate of caries attacks on buccal surfaces of maxillary incisors is known to be associated with both biological mechanisms and psychosocial behaviours²⁴. The mandibular teeth were less affected, which is consistent with the findings of a previous study²⁵.

In previous investigations, it has been reported that oral hygiene and feeding attitudes, salivary factors, as well as the level of education of the mother were related to caries development, which are essential for the initiation and progression of the disease¹⁻⁴. Furthermore, recent studies have shown a strong inverse relation between parental education and/or social background and dental health^{26,27}. Contrary to these earlier studies, in the present study no association was found between parental education and dental caries. Our results were in accordance with the study of Karjalainen et al.28. In our previous study a negative correlation was observed between maternal education and salivary S. mutans levels²⁹. In contrast, no correlation was found in the present study between maternal education and S. mutans counts of the children since the study population is limited.

Poor oral hygiene is also one of the risk factors for dental caries^{2,5,6}. It has been reported that children with poor oral hygiene have more decayed teeth, and toothbrushing is associated with a significantly reduced risk of caries⁶. No significant correlation was found between toothbrushing and dental caries scores in the present study. A significant effect of toothbrushing was not seen in this group of children, probably because of the misleading information of the parents. In the present study, a significant positive correlation was found between baseline and final dental plaque scores and final dental caries scores of the children, which was in accordance with the findings of Mattos-Graner *et al.*³⁰.

It has been reported that the use of feeding bottle was related with caries development. Although the consumption of sugar-containing beverages has been correlated with the presence of dental caries^{3,5,7,8}, this result was not confirmed in the present study. In the present study, no significant correlation was found between breastfeeding or prolonged bottle feeding and dental caries scores. The inefficacy of feeding attitudes on child's dental caries scores might be because of the mothers' knowledge about oral health. During the baseline evaluation in the present study, all mothers were informed about feeding and oral hygiene attitudes. It can be thought that efforts to educate parents to reduce sugary snacks and increase brushing are important challenges in paediatric dental care. Although the consumption of sugarcontaining beverages has been correlated with the presence of MS in infant⁸, this finding was not confirmed in this study since the subject population is limited. Further studies are needed to analyse these factors in children.

In many studies a positive correlation between the severity of caries experience and the level of MS in saliva or plaque has been shown^{4,10,11}. Although risk factors for caries were studied

extensively, only a few have studied the relationship of MS on caries progression in children who were initially caries free⁴. The results of the present study suggest that once children are identified harbouring high salivary S. mutans levels, they have a greater chance of developing caries than children with low levels of MS, since MS transmission and colonization in the oral cavity are important factors in the development of dental caries⁷. Usually the mother is regarded as the main source for the organisms found in the children^{7,12}. This finding indicates the need to focus preventive efforts on mothers with high levels of salivary MS to minimize the caries risk in their children. In the present study, a significant relationship was found between the baseline maternal sharing and the final dental caries scores. A significant correlation was also found between maternal sharing and salivary S. mutans levels. Early identification of poor oral hygiene, maternal sharing, feeding attitudes, and early colonization of caries-related microorganisms should be considered in preventive health promotion strategies. Further studies, particularly focusing on caries incidence in treated children, are necessary before a treatment protocol can be recommended for the general population.

In conclusion, the results of the present longitudinal study indicated that early *S. mutans* colonization, high plaque accumulation, and maternal sharing were found to be important factors on caries development in children. Although we have not found any correlation between feeding and oral hygiene attitudes and dental caries in the present study, further studies with larger number of subjects may clarify the association between these parameters.

What this paper adds

• The pattern of caries development was consistent with earlier studies, and evidence for the association of early colonization of *Streptococcus mutans*, maternal sharing, and dental plaque accumulation with the development of a carious lesion were reinforced.

Why this paper is important to paediatric dentists

• The early identification of poor oral hygiene, improper feeding habits, salivary parameters, and maternal sharing should be considered in preventive health promotion strategies.

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