Prevention of caries in children by preventive and operative dental care for mothers in rural Anatolia, Turkey

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Türksel Dülgergil Ç, Satici Ö, Yildirim I, Yavuz İ. Prevention of caries in children by preventive and operative dental care for mothers in rural Anatolia, Turkey. Acta Odontol Scand 2004;62:251–257. Oslo. ISSN 0001-6357.

The aim of this study was to prevent or delay the transmission of cariogenic bacteria, and hence the early development of caries in children, by preventive methods targeted at the mother. This field study was carried out in rural southeastern Anatolia, Turkey, where access to dental care is limited. Twenty-seven mother–infant pairs were followed for 2 years. The infants were between 2 and 18 months old when the study began. Mothers in the control group ($n = 12$) received a simple care and advice program, and those in the test group ($n = 15$) followed a preventive and operative regimen. The occurrence and the incremental occurrence of caries in the mothers and children in both groups were determined annually. Using a commercial kit, levels of mutans streptococci and lactobacilli in saliva and plaque were measured in the children at 6-month intervals. The microbial data demonstrated that the children of mothers in the test group had significant reductions in mutans streptococci and lactobacilli in plaque ($P < 0.001$), whereas no such trend was observed in control children during the 24-month monitoring period ($P > 0.05$). After 12 months, the occurrence of caries (dfs) was significantly lower in the test group than in the control group (0.13 ± 0.35 vs 1.67 ± 1.30, respectively; $P < 0.001$). A similar difference was observed after 24 months (0.2 ± 0.56 vs 3.17 ± 1.70, respectively; $P < 0.001$). The results of this 2-year study demonstrate that a preventive and operative regimen designed to reduce oral bacterial levels in mothers can be remarkably effective in reducing the incidence of caries in infants in rural southeastern Anatolia. Owing to the prevalence of a traditional lifestyle based on close-knit families and clans, this region is an advantageous environment for the reduction of bacterial transmission from mother to child, while largely excluding other sources of infection for the child. □ *Atraumatic restorative treatment (ART); bacterial transmission; chlorhexidine-rinsing; fluoride varnish; lactobacilli; mutans streptococci*

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Numerous clinical and microbiological studies suggest that dental caries is an infectious disease caused mainly by mutans streptococci (MS) (1). These bacteria are transmitted within the population, and genotyping has shown that similar strains of MS often colonize the members of a family (2).

Mothers seem to have a special role as a source of MS infection. Children more often share similar bacterial strains with their mother than with other family members, and children of mothers who are highly infected with MS usually show high infection levels, and vice versa (3–6). Early establishment of MS in young children is predictive of early and extensive development of caries in the primary dentition. Moreover, it has been shown in numerous studies that if a child is relatively free from caries in the primary dentition, the permanent dentition will have a minimal amount of caries (7–10).

To reduce the risk of transmission of MS from mother to child, preventive measures have been directed at reducing the salivary levels of MS in mothers (11–14). Most of these studies have been carried out in the Scandinavian countries, where preventive treatment is well organized and all pregnant mothers and newborn infants have access to maternity clinics and dental health centres (15, 16). There have been few reports of preventive measures targeting the reduction of salivary levels of MS in mothers in less developed and/or developing countries. Such research would require the use of practical and less technical approaches, given the limiting socio-economic conditions. Southeastern Anatolia in Turkey is an example of a rural region where many physical and economic limitations generally hinder extensive and effective preventive programs from being put into practice. In addition, families are organized within large tribal units and the agricultural economy creates an isolated lifestyle in the rural districts. As a result of massive irrigation projects implemented by the government, the majority of the people are field-workers, particularly in cotton fields. Accordingly, there is a close relationship among members of the same clan and this creates an interesting environment in which newborns are in contact almost exclusively with their mothers and siblings until they are 4 to 5 years old, and can live in the same town for a long time without external interactions.

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DOI 10.1080/00016350410001685

As part of the oral health plan, it was decided to carry out a pilot study assessing the applicability and activity of a preventive measure targeting mothers with newborn infants in rural Anatolia. The null hypothesis to be tested was that there is a considerable anticaries effect on the primary dentition of children living in rural districts when their mothers are subjected to preventive and/or operative measures at the time the first tooth emerges. Therefore, in this 2-year study, we investigated whether preventive measures targeting the reduction of maternal salivary MS and LB levels could also reduce the colonization of these organisms in children, and thus decrease the risk of infant caries in a rural region where the availability of alternative preventive approaches is limited owing to high costs.

Materials and methods

Subjects

The study was carried out in a small village near the city of Diyarbakir in southeastern Anatolia, Turkey. In this region, oral health care is difficult to obtain in most rural areas, and, when available, tooth extraction is the predominant mode of treatment. As in many other rural districts of Turkey, limited financial resources and widespread settlement tracts have resulted in community-oriented caries-prevention projects being ignored for many years.

The demographic structure of this village was characterized by large clans, with many individuals having relatives in different clans. Mothers with newborn children were contacted and examined during house-to-house visits. Owing to the traditional lifestyle, with extended families within one large clan sharing a large home enclosed by a garden, each house visit permitted contact with a number of mothers and their children. The vast majority of the mothers examined had never received dental care, and none of them had undergone restorative care except tooth extraction. The level of oral hygiene was thus fairly low—the mothers generally with little knowledge and poor attitudes and habits pertaining to oral health.

Of the 53 mother–child pairs examined, 27 were enrolled in the study, having met the following selection criteria: (i) residence in the same village and, but not a prerequisite, membership in the same tribal group; (ii) willingness of both the mother and her husband to sign a statement of informed consent allowing inclusion of the infant in the research; (iii) no pregnancy in the previous 6 months; (iv) any carious teeth of the mother were viable and treatable with vital operative treatment; (v) the infant was the youngest child of his or her family; (vi) the infant was in good general health; (vii) the infant had not received any systemic or local antibiotics or local antimicrobial medication in the previous 6 months; (viii) the infant did not have more than 6 teeth already erupted; and (ix) the infant did not have any cavitated–carious lesions. The mothers selected for the study were between 20 and 37 years of age (27.07 ± 4.01, mean $\pm$ *s*) and their infants were 2 to 18 months (8.3 ± 4.0 months).

Sampling of saliva and plaque, and enumeration of cariogenic bacteria

Paraffin-wax-stimulated saliva was obtained before breakfast for each measurement period. Saliva samples were collected in a sterile tube by spitting, and were spilled onto a specifically designed agar-test surface for microbial investigation (CRT Bacteria—Two in One System, Vivacare Line, Vivadent, Lichtenstein). These commercial test kits employ a selective medium for the cultivation of MS and LB. After 48 h incubation at 37°C in a specially designed incubator, the density of adherent stained colonies on the strips was visually graded using calibration cards provided by the manufacturer. No colony recognition was required. In infants, MS and LB levels in plaque samples were also determined using a site-specific method described by Bratthall et al. (17). In this procedure, a sterile wooden toothpick was used to harvest the plaque samples from the buccal sides of maxillary primary incisors. The toothpicks were then used to seed the pad of the test strips, and the incubation and interpretation of results was as for the saliva samples. The result was assigned to an MS class, generally 0, 1, 2, or 3, corresponding approximately to bacterial concentrations of 10^3, 10^4, 10^5, or 10^6 CFU/mL of plaque. All sampling and scoring procedures were carried out by one author blinded to the treatment groups (IY). The 27 mother–child pairs were then randomly divided into a test group ($n = 15$) and a control group ($n = 12$).

Dental examination and treatment plan

The dental examinations were carried out by one author (ÇTD) using mirror, probe, and sunlight for illumination. The World Health Organization (WHO) criteria for caries were followed for assessment of mothers (DMF-T) and infants (df-s). All cavitated lesions were recorded and included for calculations in all mother–child pairs. If there was any doubt, caries was not recorded.

In cases where the crown had been destroyed by decay and only the root was left, the root remnants were extracted if not prohibitively difficult to do so in the rural conditions; consequently, these cases were recorded as missing (M) components only. In addition, and if necessary, simple tooth extractions were performed under local anesthesia, usually in the garden of the extended family's home, as dictated by the rural conditions. A simple scaling procedure was performed for all mothers, followed by a 3% hydrogen peroxide mouthwash to stop gingival bleeding.

The control group mothers received only a simple care and advice program, including oral hygiene instructions through a detailed demonstration of tooth-brushing with a fluoridated toothpaste (İpana floristat, Eczacıbaşı-Procter

and Gamble Co., Istanbul, Turkey), and restoration, if possible, of the cavitated lesions using the atraumatic restorative treatment (ART) technique (Ketac-Molar, ESPE, Germany).

The mothers in the test group were given a detailed preventive and operative program consisting of: (i) oral hygiene instruction including detailed demonstration of a tooth-brushing procedure with a smooth brush (İpana aktif, Eczacıbaşı-Procter and Gamble Co.) and a fluoridated toothpaste including triclosan (Colgate-TOTAL, Colgate-Palmolive Co., Istanbul, Turkey), (ii) treatment of dental caries by the ART technique using a high-strength glass-ionomer cement (Ketac-Molar), (iii) professional flossing followed by application of a fluoride varnish (Fluor Protector, Vivadent/Vivacare, Schaan, Liechtenstein) twice per year, (iv) sealing of occlusal pits and fissures with a glass-ionomer cement (GIC) if discolored and deep fissures were present after tactile examination with a dental probe, and (v) twice daily rinsing with 0.02% chlorhexidine (CHX) (Klorhegs, Drog-San, Ankara, Turkey) for 10 days, and repetition of this procedure twice per year (Table 1). In contrast to many similar studies targeting mother–child pairs, mothers were not asked to change their dietary habits during the study.

The effect of the treatment was monitored by microbiological testing of saliva samples from the mothers, and plaque samples from the children, using the test kits mentioned above. For the mothers, saliva samples were collected every 6 months for 24 months, with the addition of a sample taken 3 months after examination to determine the one-time effect of the basic preventive and operative measures. For the children, bacterial levels were measured in plaque every 6 months. The children were also examined annually for the occurrence of caries by a second experienced clinician (IzY) blinded to treatment groups and bacterial findings. Inter-examiner reliability was determined using examination results from 5 test and 5 control mothers at the start of the study, for a kappa of 0.95, and again using examination results from 5 test and 5 control children in both month 12 and month 24, yielding kappa values of 0.95 and 0.93, respectively.

Table 1. A preventive and operative intervention applied to test mothers during the study

Time period
Selection of mother–infant pairs having inclusive criteria.
Baseline
Saliva samples from mothers and plaque samples from infants if teeth were present.
Recording of caries\mother, count of teeth\infant.
Application of preventive regimen including:
ART fillings\sealants, hygiene instructions, scaling, F varnish
2 × daily CHX rinsing during 10 days (periodically).
3rd month
Saliva samples from mothers.
2 × daily CHX rinsing during 10 days.
6th month
Samples from mothers, and plaque samples from infants.
2 × daily CHX rinsing during 10 days.
12th month
Saliva samples from mothers and plaque samples from infants.
Recording of caries for mother–infant pairs, count of infant teeth.
2 × daily CHX rinsing during 10 days.
F varnish and, if necessary, replacement of fillings and sealants.
18th month
Same as in 6th month.
24th month
Saliva samples from mothers and plaque samples from infants.
Rcording of caries for mother–infant pairs.

Statistical analysis

Spearman's rank correlation test was used to explore the correlations between the variables in the test and control groups. The median values of bacterial scores in saliva and plaque samples for each group and each measurement period were compared by Friedman ANOVA analysis. The relationships between the baseline-to-12-month and baseline-to-24-month dft and dfs values of each group were examined using the Wilcoxon matched-pairs signed rank test. Comparisons between the test and control groups of children and mothers with regard to the microbiological variables, and dfs and dft values, were made by Mann-Whitney U test. A difference at the level of $P < 0.05$ was regarded as significant.

Results

At the end of the 2-year follow-up period, 2 of the 15 infants in the test group developed a total of 3 caries lesions, while of the 12 infants in the control group, 11 developed a total of 24 caries during the study period. Comparing both groups with respect to the number of caries-free children there was a statistically significant difference (87% vs 8%, $P < 0.001$). The occurrence of caries, as measured by mean dft and dfs, was significantly lower in the test children than in the control children ($P < 0.001$; Table 2). The difference in the mean caries increment was statistically significant ($P < 0.005$ for dft and $P < 0.001$ for dfs). The changes from 12 to 24 months in dft (Δ dft or dft_{24}–dft_{12}) and dfs (Δ dfs or dfs_{24}–dfs_{12}) for the control group were nearly 15 and 25 times greater than those of the test group, respectively ($P < 0.05$).

The distribution of MS and LB scores differed between the test and control children in all examinations after the preventive measures were started (Figs 1, 2). At the end of the study period, 5 out of 15 test children had an MS score for plaque samples of 0 (range 0 to 1), while in the control group none of the children had MS scores of 0 in either sample (median score 3, range 2 to 3). The median values of MS and LB scores were significantly lower in the infants in the test group at each sampling time, and particularly at 24 months; the difference was the same for plaque levels of MS and LB ($P < 0.001$).

Changes in salivary MS and LB levels in mothers during the course of the study are illustrated in Figs 3 and 4. The decreases in both salivary MS and LB levels in test

Table 2. Baseline age and number of teeth, and dft values of test and control infants in 12th and 24th month examinations

Group	Age at baseline* (mean ± *s*)	No. of teeth at baseline (mean ± *s*)	dft (mean ± *s*)		dfs (mean ± *s*)	
			12th mo	24th mo	12th mo	24th mo
Test	8.4 ± 4.06	2.06 ± 2.15	0.13 ± 0.35	0.2 ± 0.56	0.13 ± 0.35	0.2 ± 0.56
Control	8.2 ± 4.0	2.1 ± 1.3	1.08 ± 0.79	2.0 ± 1.2	1.66 ± 1.3	3.16 ± 1.69

* Age as month.
s = standard deviation.

mothers, from the first to final measurements, were found to be statistically significant (Friedman ANOVA; $P < 0.001$ for both MS and LB). Compared to the control mothers, test mothers had significantly lower MS and LB values for each sampling time, beginning at month 3 ($P < 0.01$). While at the time of baseline assessment the caries experiences of the test and control mothers were similar ($P > 0.05$), there was a slight but statistically significant difference at the end of the study ($P < 0.01$; Table 3).

Discussion

The results of numerous field trials have provided emphatic evidence that the reduction or elimination of highly cariogenic microflora in the population can be an important strategy for the prevention of caries. A logical alternative to eliminating the cariogenic flora in infected patients is to prevent or postpone infection for as long as possible. Consistent with this suggestion, clinical data show that the later a child is infected with MS, the fewer caries it will develop (7, 18). Therefore, efforts have been made to prevent or delay the transmission of MS from mother to child, beginning as early as possible.

Although most preventive intervention studies in mothers and children have started when the children were 6 to 9 months old, and a few have started with expectant mothers, almost all of them have shown a lower incidence of early childhood caries. Recently, Brambilla et al. (19) studied the use of a minimal preventive regimen during pregnancy. The authors' objective was to explore the effectiveness of a caries preventive regimen that included daily rinsing with 0.05% sodium fluoride and 0.12% chlorhexidine in pregnant women, and subsequently in their children during a 30-month period. They showed that the treatment significantly reduced the salivary MS levels in the mothers and delayed colonization of the children with MS bacteria by about 4 months. Gunay et al. (20) used a pre- and postnatal prevention program to improve the dietary habits of mothers and to prevent or delay the transmission of MS. Their results showed a marked improvement in the oral health of the mothers and a significantly lower level of dental caries in the children after 4 years. Further evidence supporting the initiation of

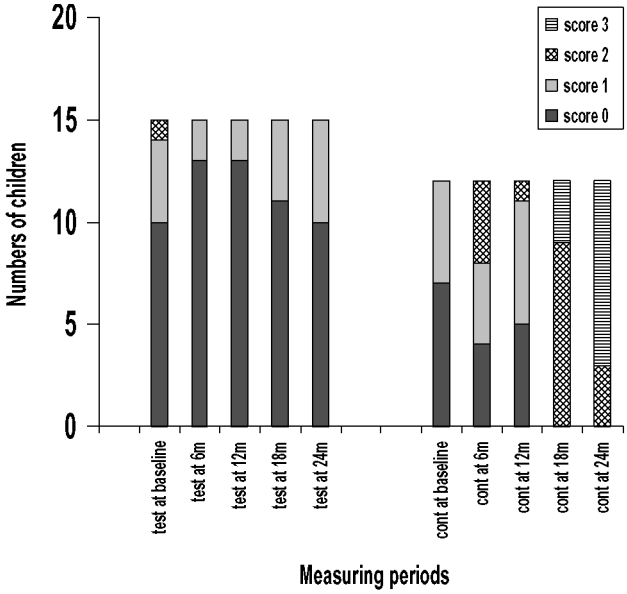

Fig. 1. For each sampling period [baseline, 6th-12th-18th and 24th month], distribution of MS scores according to numbers of test and control infants.

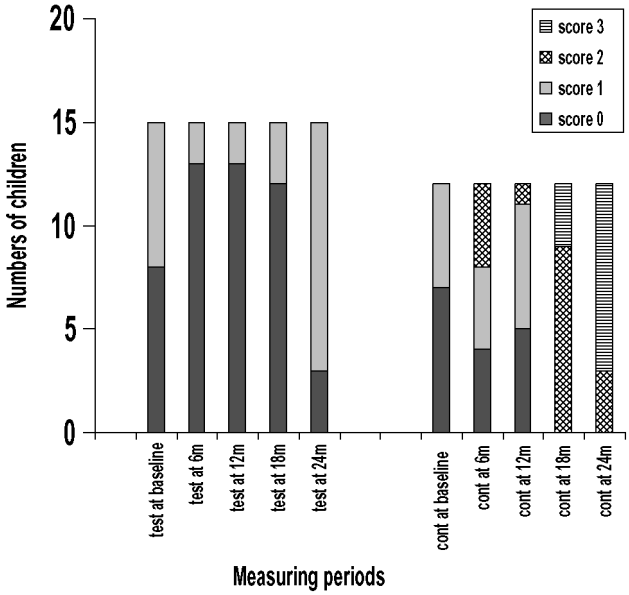

Fig. 2. For each sampling period [baseline, 6th-12th-18th and 24th month], distribution of LB scores according to numbers of test and control infants.

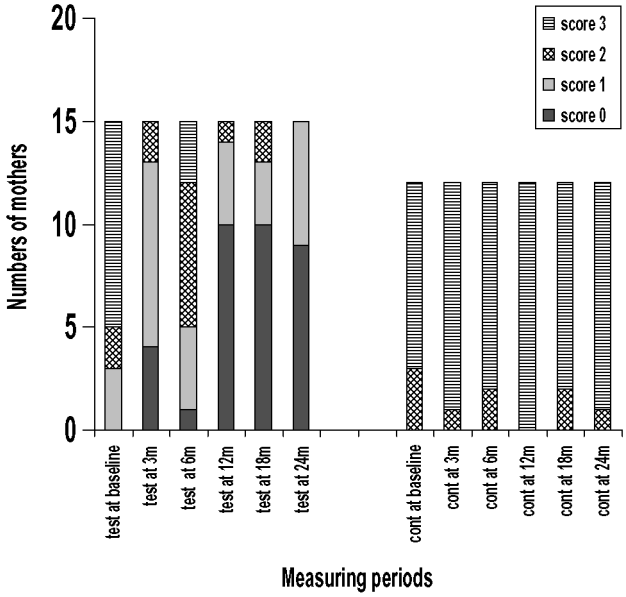

Fig. 3. For each sampling period [baseline, 3rd-6th-12th-18th and 24th month], distribution of MS scores according to numbers of test and control mothers.

caries prophylactic measures in pregnant women has come from a study of a mother–child caries preventive program by Gomez et al. (21), who reported that after 4 years 97% of the children were caries-free as compared to 77% in the control group. Tenovou et al. (22) stated that biannual treatment of mothers with a combination of 1% CHX and

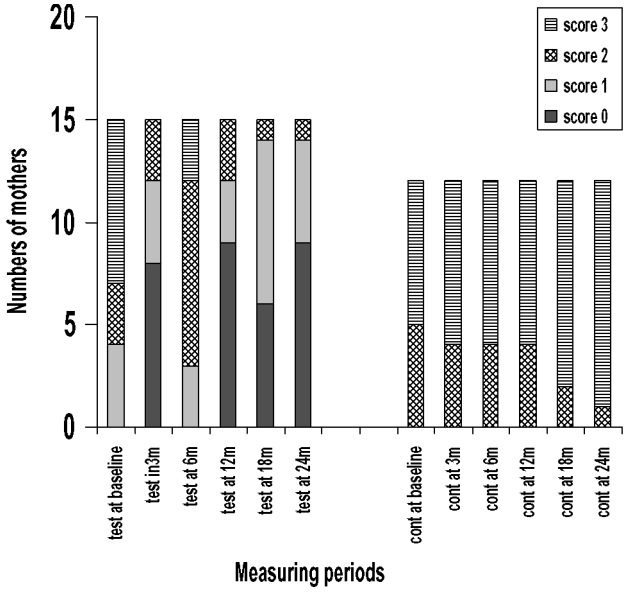

Fig. 4. For each sampling period [baseline, 3rd-6th-12th-18th and 24th month], distribution of LB scores according to numbers of test and control mothers.

Table 3. DMFT index and age of mothers in the test and control groups

		DMFT (mean ± *s*)	
Group	Age (mean ± *s*) (year)	Baseline	24th month
Test	27.0 ± 4.17	5.0 ± 2.07	5.13 ± 2.13
Control	27.16 ± 3.97	5.33 ± 4.0	7.25 ± 3.84

s = standard deviation.

0.2% fluoride gel for a period of 3 years reduced bacterial colonization of the infants significantly.

In these studies, however, the differences in the occurrence of caries in children whose mothers received or did not receive preventive interventions ranged 1.5-fold to 7-fold. Interestingly, the present study demonstrated that a preventive dental program used in a rural district was clearly more effective than those performed in city centres, as reported in previous studies. At the 24-month follow-up in our study, the dfs was 0.2 for children in the test group and 3.16 for children in the control group. Although our study group is too small for a scientific conclusion to be reached, this surprising difference, with an approximately 15-fold higher rate of carious surfaces seen in the control group, has been found to be statistically significant. Currently, the traditional tribal lifestyle in rural south-eastern Anatolia may offer a number of advantages to preventive dental care programs, leading to a long-lasting and marked effect on caries prevention in children.

It has been confirmed previously that infants are more susceptible to acquiring MS during a window of infectivity ranging from 19 to 31 months of age (2, 18). However, transmission is not limited to this window, since mothers harbor a more heterogeneous population of MS than their children, suggesting that additional strains are acquired as children grow up (18). Nor is transmission limited to the mother–child relationship in modern lifestyles. Any caretaker can transmit infection, as can playmates at daycare centres if they share toys or utensils that are placed in the mouth. Consequently, it is hard to apply preventive programs such as the one presented in the current study to all infants, especially those living in city centres; to be effective this approach may need to include other family members, such as fathers, siblings, or grandparents, if they care for the infant. However, since babies in rural south-eastern Anatolia are cared for almost exclusively by their mothers, this difficulty was avoided.

One factor influencing our findings was the traditional lifestyle that is still practised. Children's interactions are limited to family members, and children are commonly in contact with their mothers until the age of six, when they start primary school. This situation may hinder, or even prevent, exposure of children to external sources of bacterial contamination, with the exception of their mothers. Thus, tribal living in a large village home, or in several homes close to each other, may provide a strictly limited environment for bacterial transmission, wherein transmission occurs routinely from mother to infant, but

not from any individual outside the immediate members of the tribal unit.

A second consideration in the interpretation of our results is that even though continuously high bacterial transmission from mother to child routinely occurs, it is possible that the lower increment of caries seen in the children in the test group was due to the prevalence of fewer cariogenic MS strains after use of the preventive regimen, especially one that included rinsing with CHX. Such a possibility was recently confirmed in a 6-year follow-up study showing that, despite high bacterial transmission from mother to child, a lower occurrence of caries was observed in children whose mothers used xylitol chewing gum (23). Even years after cessation of habitual xylitol consumption, the mothers harbored and transmitted low-adherence MS strains due to the effects of the xylitol treatment on bacterial populations. Similarly, the effects of CHX treatments on oral flora, including MS, are believed to last for at least 1 to 3 months after treatment (22, 24, 25). In one of the most ambitious studies, Etemadzadeh et al. (26) showed an approximately 1.1 log decrease in both MS and LB counts after a CHX rinsing program, resulting in a 44% decrease in the risk of caries. Consistent with this result, our study found that chlorhexidine rinse applied periodically resulted in an efficacious reduction in oral bacteria in mothers in the test group. However, in spite of antimicrobial treatment, it has not been possible to eradicate MS completely from the oral cavity for any extended period, especially when retentive sites are present in the dentition (27). Consequently, in the present study, before antimicrobial treatment was initiated, restorations and sealants were placed to reduce the number of retentive sites and eliminate the niches colonized by cariogenic bacteria such as MS and LB. Notwithstanding that some test mothers had MS scores in saliva of 0 in the current study, this does not necessarily indicate that MS was completely removed from the mouth. This should be interpreted as an indication that the interventions applied to mothers during the 2-year period had reduced bacterial levels below the limits of detection of the strip method.

In addition to the MS scores in the mothers, other factors may also affect the mother–child transmission of MS, such as the infants' feeding practices (28) and the dietary habits of the family, such as sucrose consumption by the parents (29), because a diet with limited sugar content is not conducive to implantation of MS (30, 31). Although, in general, dramatic dietary changes are necessary for a reduction in MS (32), the already low sucrose consumption seen in rural southeastern Anatolia, combined with the preventive intervention, might have favored the surprisingly low prevalence of caries seen in the test-group children.

In contrast to the findings in the test-group mothers, it is possible that the high number of untreated caries lesions that occurred during the study in the mothers in the control group may have served as an important source of transmission of MS to their children. Furthermore, the unexpected increase in the LB score in control-group mothers could be explained by the increase in caries lesions, because LB primarily inhabits dental pits and fissures, as well as deep carious lesions (33, 34). Although the reduction in LB in the test group may have resulted from the removal of diseased tissue that was laden with these bacteria, improvement in the participants' ability to cleanse the dentition, alteration of the microbial habitat, or some combination of these and other factors could be responsible for the observed dramatic reduction (27, 35). In this respect, although the treatment of the carious lesions and the promotion of individual oral hygiene practices at the beginning of the study were effective in reducing salivary and/or plaque bacterial scores in mothers, subsequent decreases in patient compliance, especially as regards brushing with fluoridated toothpaste, could lead to unexpected increases in LB scores over time. Although this was not systematically reported in our results, patient compliance with the use of dentifrice and toothbrushes was clearly lower among the control-group mothers than among the test-group mothers. Unfortunately, this is probably due to the frequent contact and reinforcement with the test-group mothers in the course of re-application of the preventive interventions during the study (Hawthorne effect), which was not the case for the control-group mothers. This lack of reinforcement and, hence, compliance could have led to the concomitant increases in LB scores and carious lesions in the control group.

Alternatively, one of the reasons behind the increased bacterial levels in the control group could have been the application of glass-ionomer restorations with the ART technique. Although, in a recent in vivo study, Carvalho and Bezerra (36) showed a significant reduction of MS levels in saliva on comparing the results before treatment with those obtained 1 week ($P = 0.003$), 4 weeks ($P = 0.000$), and 1 year ($P = 0.002$) after ART restorations; previous studies had clearly confirmed that the effect of fluoride release from GICs is dependent on various factors, including salivary flow rate, saliva volume, and the number of fillings placed (37, 38) in adults. Consequently, GICs placed in adults cannot be effective in reducing cariogenic bacteria in the long term, especially when oral hygiene is inadequate.

People in most villages in southeastern Anatolia have a self-sufficient way of life, with no apparent need for outside interactions. This interesting lifestyle eliminates external sources of infection for children and could create an advantageous environment for the reduction of bacterial transmission from mother to child. Also, somewhat surprisingly, this traditional lifestyle did not cause the loss of any mother–infant pairs to follow-up; all the children and mothers could easily be found whenever we returned for assessments.

The results of the present study confirm the findings of previous studies demonstrating that reduction or elimination of highly cariogenic microflora in the population can be an important strategy for the prevention of caries in

children. Accordingly, it can be asserted from this clear evidence that early preventive interventions with mothers may cause a more pronounced decline in the future caries experience of infants in rural districts than in their city-dwelling counterparts. The evidence presented here also confirmed the hypothesis that if MS is not established in the dental plaque during the first 2 years, there is minor development of caries in the early primary dentition.

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Received for publication 17 March 2004
Accepted 10 August 2004