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Influence of Maternal Xylitol Consumption on Mother-Child Transmission of Mutans Streptococci: 6-Year Follow-Up

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Key Words

Chlorhexidine varnish · Colonization · Fluoride · Mutans streptococci · Transmission · Xylitol

Abstract

Xylitol is effective as a noncariogenic or even cariostatic sugar substitute. Habitual xylitol consumption appears to select for mutans streptococci (MS) which shed easily into saliva from plaque. We have earlier shown that habitual xylitol consumption of mothers was associated with a statistically significant reduction in the probability of mother-child transmission of MS assessed at 2 years of age. The aim of the present study was to assess the children's MS counts 1 and 4 years after the maternal xylitol consumption had been discontinued. At baseline, during pregnancy, all mothers (n = 195) showed high salivary levels of MS. The mothers were randomly assigned to xylitol, fluoride (F) and chlorhexidine (CHX) groups. In the xylitol group, the mothers chewed xylitol-sweetened gum, for 21 months, starting 3 months after delivery. In the two control groups, the mothers received CHX or F varnish treatments at 6, 12 and 18 months after delivery. At the 2-year examination, 169 mother-child pairs participated. At the 3-year and 6-year examinations, there were 159 and 147 children in the study, respectively. For children's MS analyses, visible plaque was collected using toothpicks at the age of 3 and paraffin-stimulated saliva at the age of 6. The persons involved in the collection and analysis of the microbiological samples were blinded as to the study design and group. Both the plaque and salivary MS were cultured on Mitis salivarius agars containing bacitracin. In all groups, the colonization percentages increased during the follow-up. At the 3-year examination, the children's risk of having MS colonization was 2.3-fold in the F group (95% CI 1.3–4.2) compared to the xylitol group. This difference was statistically significant. Even at 6 years of age, the salivary MS levels were significantly lower in the xylitol group than in the other groups (ANOVA, $p<0.001$). In conclusion, the earlier demonstrated, xylitol-associated reduction in the probability of mother-child transmission of MS was still found in the children's MS counts at the age of 3 and 6 years.

Children whose teeth are colonized by mutans streptococci (MS) at an early age show higher caries experience than those colonized later or not at all [Alaluusua and Renkonen, 1983; Köhler et al., 1984; Tenovuo et al., 1990]. Most children appear to acquire these microorganisms from their mothers [Berkowitz and Jordan, 1975; Caufield et al., 1985, 1988]. The acquisition of MS has been suggested to occur between 19 and 31 months of age [Caufield et al., 1993], although extensive MS colonization has also been described at a younger age [Berkowitz et al., 1980; Mohan et al., 1998].

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Xylitol shows specific MS-inhibiting effects [for review see Trahan, 1995], suggesting permanent reductions in oral MS levels. In short-term habitual consumption, xylitol reduces the levels of MS assessed from both plaque and stimulated whole saliva [Loesche et al., 1984; Söderling et al., 1989]. Habitual long-term xylitol consumption appears to select for natural mutant cells of MS which are 'xylitol-resistant' [Trahan and Mouton, 1987]. Xylitol-resistant mutants appear to shed more easily into saliva from plaque than do xylitol-sensitive parental strains [Söderling et al., 1991; Trahan et al., 1992]. We have reported earlier that habitual xylitol consumption by mothers was associated with a statistically significant reduction in the probability of mother-child transmission of MS assessed at 2 years of age. In the 2-year study, the mothers regularly chewed xylitol-sweetened gum for 21 months, starting 3 months after delivery, or they received either chlorhexidine (CHX) or fluoride (F) varnish treatments at 6, 12, and 18 months after delivery. We suggested that the MS of the mothers who regularly consumed xylitol had impaired adhesion properties, leading to a reduced mother-child transmission of MS [Söderling et al., 2000]. The present study reports the MS counts of the children of the above study at the age of 3 and 6 years.

Materials and Methods

Subjects and Study Design

The study was carried out at Ylivieska Health Center in central Finland. In the health center, the mothers and children participated in a free postnatal oral health care program, which included examinations, advice on diet, oral hygiene and use of fluorides and, where necessary, restorative treatment.

For the transmission study, a total of 338 pregnant women were screened. Altogether 195 women showed high levels of MS in their saliva (CFU ≥ 105/ml), and were invited to participate in the study. Of those, 120 were randomly assigned to the xylitol gum group, 32 to the CHX treatment group, and 36 to the F group. In addition, 7 mothers who reported use of xylitol chewing gum on a daily basis, were assigned to the xylitol group. All the analyses in the present study were carried out also with the children of these 7 mothers excluded. These 7 children in no way differed from the other children in the xylitol group at any annual examination. Therefore, they were included in all the results. For the details of the randomization and study design, see Söderling et al. [2000]. The mothers received written information about the study design (table 1) and the samples to be collected, and they were specifically instructed not to change their dietary habits during the study. The subjects participated after giving their informed consent to the study protocol. The protocol was reviewed and approved by the Ethical Boards of both the Medical Faculty at Turku University and the Ylivieska Health Center.

In the xylitol group, the mothers were recommended to chew xylitol gum at least 2–3 times a day. The gum use started when the children were 3 months old and continued until they were 2 years old. The chewing gum contained xylitol as the only sweetener (65% w/w) and it was supplied for the study by Leaf (Turku, Finland). In the CHX and F groups, the mothers received CHX or F varnish treatments at 6, 12 and 18 months after delivery. The varnish treatments (CHX varnish: EC 40, Certichem, Nijmegen, The Netherlands; and F varnish: Duraphat®, Rhône-Poulenc Rorer GmbH, Köln, Germany) were performed as described earlier [Söderling et al., 2000]. The children's teeth were not treated with varnish and no gum was given to the children before the age of 2 years. All the children, regardless of the study group and regardless of the possible caries risk, got an oral health care program which has been routinely given to children under 5 years of age in the Finnish public health care system, including regular examinations, advice on diet, oral hygiene and use of fluorides and, where necessary, restorative treatment.

At the child's 2-year examination at the end of the intervention period, the groups consisted of 169 mother-child pairs (106/xylitol, 33/F, 30/CHX). At the 3-year examination, there were 159 (98/xylitol, 33/F, 28/CHX) mother-child pairs in the study. Only the children (n = 147; 93/xylitol, 31/F, 22/CHX) provided samples at the 6-year examination. However, the mothers accompanied the children and were interviewed. At both the 3- and 6-year examinations, the mothers were interviewed about type of daycare, changes in the diet, acute and chronic illnesses of the child, medication of either the mother or the child, oral hygiene procedures, and use of xylitol and F by the mother or the child. The main reason for subjects interrupting the study was the family moving away from the area. The mothers in the three groups were comparable at baseline (age, DMFT) and the dropouts did not differ significantly from those who completed the study.

The distribution of boys and girls was similar in all three groups. There were no differences among the groups in the number of children's teeth at 6 months, 1 or 2 years of age. In the xylitol and F groups, the dropouts were distributed similarly between the subgroups with and without MS colonization. However, at the 6-year examination, all dropouts from the CHX group were in the subgroup without MS colonization.

Table 1. Study design used for comparison of the effect of xylitol consumption with F or CHX varnish applications on mother-child transmission of MS

Pregnancy	Selection of pregnant women with high levels of salivary MS[a]
Delivery	
Child 3 months	The use of xylitol starts in xylitol group
Child 6 months	F or CHX varnish treatment of the mother in F and CHX groups
Child 1 year	F or CHX varnish treatment of the mother in F and CHX groups
Child 1.5 years	F or CHX varnish treatment of the mother in F and CHX groups
Child 2 years	Pooled plaque sample from the child. Use of xylitol discontinued in xylitol group
Child 3 years	Pooled plaque sample from the child
Child 6 years	Saliva sample from the child

[a] The salivary MS levels of the mothers were also assessed at the child's age of 6 months, 1 year, 2 and 3 years.

In the rural area where the study was carried out the diet is rather uniform among families with small children. The interviews at the 3- and 6-year examinations revealed no differences between the three study groups.

Plaque and Saliva Sampling

The mothers were requested not to brush their own or their child's teeth on the morning of the saliva sampling at the 3-year examination. They were also instructed not to eat or drink for at least 1 h before the appointment. The same instructions concerning the children were given to the mothers who accompanied their children at the 6-year examination.

Eight experienced dentists collected the plaque and saliva samples. They were blinded with regard to the study design and study group of the child/mother, as well as to the MS colonization of the child. Visible plaque was collected using toothpicks [Tenovuo et al., 1992]. Two children would not cooperate in plaque collection, and thus no sample was obtained. The plaque was collected from all tooth surfaces, particularly from the cervical margins and interproximal regions, with 1–4 toothpicks, the tips of which were cut off and pooled in 0.5 ml transport medium containing tryptic soy broth (Difco, Detroit, Mich., USA) with 10% glycerol (v/v). The number-coded transport tubes were stored frozen at –70 °C before microbiological analysis. The mothers provided 2 ml of paraffin-stimulated whole saliva at the 3-year appointment, and the children 2 ml of saliva at the 6-year appointment. Thereafter, 100 µl of saliva was immediately transferred into 900 µl of transport medium and stored frozen until microbiological analyses.

Microbiological Analyses

The microbiological analyses were performed by a laboratory assistant and an experienced microbiologist. Both were blinded as to the group of the child/mother. The study code was broken after all 3-year samples had been analyzed. The 6-year samples were collected and analyzed using a different code and without access to the results of the earlier examinations. For the microbiological analyses of plaque and saliva the transport tubes were thawed and vortexed thoroughly for 1 min. To detach the plaque from the toothpicks and disrupt bacterial aggregates, the plaque samples were additionally treated with mild 10-second sonication at +4 °C (100-watt sonicator: MSE, London, UK; amplitude 3, end diameter of the probe 3 mm). Our earlier studies [Trahan et al., 1992] had shown that the MS levels are not affected by the laboratory methods used in this study. After serial 10-fold dilutions, the bacteria were plated on Mitis salivarius agars containing bacitracin (MSB) [Gold et al., 1973]. The plates were incubated for 2 days in a 7% CO_2 atmosphere at 37 °C. MS were identified on the basis of colony morphology and counted using a stereomicroscope. The identification of *Streptococcus mutans* and *Streptococcus sobrinus* was performed as described earlier [Fujiwara et al., 1991]. Classification of *S. mutans* was based on consistent findings of 'rough' colony morphology on the MSB plate, positive fermentation with sorbitol, mannitol, raffinose and melibiose, and negative dextran agglutination. The identification of *S. sobrinus* was based on 'smooth' colonies on the MSB plate, positive fermentation with mannitol but negative fermentation with raffinose and melibiose, and positive dextran agglutination. For saliva, the detection limit of the MS assay was 250 CFU/ml, and for plaque, 20 CFU/sample. The plaque samples were also cultured on blood agars to ensure that a substantial amount of plaque had been obtained in the plaque collection.

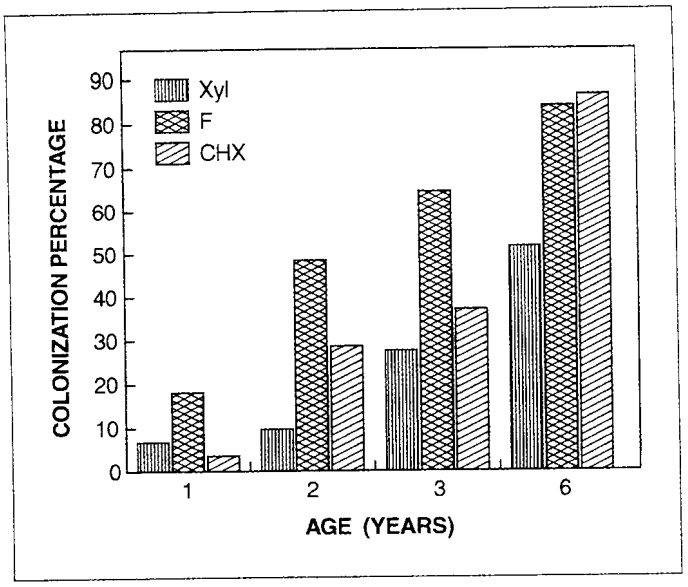

Fig. 1. The percentage of children showing detectable colonization with MS at 1, 2, 3 (plaque MS; n = 159) and 6 (salivary MS; n = 147) years of age. Groups: F = fluoride varnish, CHX = chlorhexidine varnish, Xyl = xylitol chewing gum. For statistical significances, see text. The results for the 1- and 2-year-old children have been reported earlier [Söderling et al., 2000].

Statistical Analyses

Analysis of variance (ANOVA) was used for comparison of salivary MS levels of mothers and 6-year-old children among the groups, and Student's t test for comparison of dropouts (DMFT). The proportion of children in each group with detectable levels of MS in plaque at the 3-year examination and in saliva at the 6-year examination was used as the measure of transmission. The difference between the groups was analyzed using relative risk (RR) [Rothman, 1986]. The χ^2 test was used to analyze the association between usage of xylitol or fluorides, daycare type, or antibiotic usage and MS colonization in children. The level of statistical significance was set at $p<0.05$. Statistical computing was carried out with the CSS: Statistica program.

Results

The colonization percentages increased in all groups during the follow-up (fig. 1). At 3 years of age, 27.6% of the children in the xylitol group were colonized with MS. The corresponding figures were 64.5% in the F and 37.0% in the CHX group (fig. 1). Thus, the children's risk of MS colonization was 2.3-fold in the F group (95% CI 1.3–4.2) compared to the xylitol group. This difference was statistically significant. The probability of MS colonization did not differ between CHX and xylitol or CHX and F groups. At the 6-year examination, 51.6% of the children in the xylitol, 83.9% in the F and 86.4% in the CHX group showed detectable MS counts in their saliva samples (fig. 1). The dif-

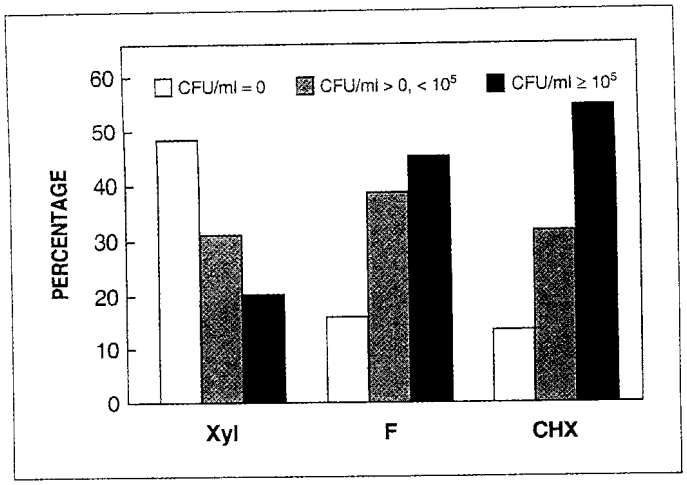

Fig. 2. The percentage of children showing no, low (CFU/ml $< 10^5$) or high (CFU/ml $\geq 10^5$) salivary MS counts at 6 years of age. Groups: F = fluoride varnish, CHX = chlorhexidine varnish, Xyl = xylitol chewing gum. The salivary MS levels were significantly lower in the xylitol group than in the other groups (ANOVA, $p<0.001$).

ference between the xylitol and F groups was statistically significant (RR: 1.6; 95% CI 1.0–2.6). The salivary MS levels were significantly lower in the xylitol group than in the other groups when the percentages of children showing no, low ($<10^5$ CFU/ml) or high ($\geq 10^5$ CFU/ml) salivary MS were compared (ANOVA, $p<0.001$, fig. 2).

At the 3-year examination, the levels of salivary MS (CFU/ml ± SD) of the mothers during the study were 5.4 ± 1.2 in the xylitol, 5.3 ± 1.2 in the F and 5.9 ± 0.7 in the CHX group. The differences were not statistically significant. Less than 10% of the mothers in the three groups showed detectable levels of *S. sobrinus* in their saliva, with no significant differences among the groups. None of the children was colonized with *S. sobrinus* at 3 years of age. At 6 years, the *S. sobrinus* colonization percentages were below 10% in all groups.

No significant associations between MS colonization and type of daycare or usage of antibiotics or fluorides by either the mother or the child were detected. At 6 years of age, approximately one third of the children in the three groups chewed xylitol gum on a nondaily basis. The rest of the children chewed xylitol gum on a weekly basis or infrequently. Only a small percentage of the children did not use xylitol products at all. The usage patterns did not differ among the groups.

Discussion

This is the third report of a longitudinal study exploring the effects of xylitol consumption by mothers on mother-child transmission of MS, and on the children's risk of future caries development [Söderling et al., 2000; Isokangas et al., 2000]. We have earlier reported that regular xylitol consumption by mothers drastically reduced the probability of mother-child transmission of MS when assessed at 2 years of age [Söderling et al., 2000]. The present results show that the probability of MS colonization, as well as of high salivary MS counts was reduced in the xylitol group in comparison with the F and CHX groups. The children in the xylitol group showed reduced MS counts for as long as 4 years after the cessation of the maternal xylitol gum consumption. Thus, our results are in accordance with earlier findings demonstrating that a reduction in the mothers' MS counts results in long-term reduction in MS colonization of their children [Köhler and Andréen, 1994]. No other factors explaining this finding, for example, xylitol consumption of the children themselves or differences in the maternal salivary MS count among the groups, were detected. We have also shown that the presence of MS at the age of 2 years was related to the child's age at the first caries attack. At 5 years of age, the occurrence of dental decay in the xylitol group was reduced by about 70% as compared with the two comparison groups [Isokangas et al., 2000].

In our study, the interventions were discontinued when the children were 2 years of age. At present, there is disagreement in the literature as to whether children are colonized during a discrete 'window of infectivity' [Caufield et al., 1993] or both before and after this 'window' [Berkowitz et al., 1975; Mohan et al., 1998; Straetemans et al., 1998]. The chosen intervention period appears to coincide closely with the general period of infectivity. The present findings, however, do not support the idea of a discrete 'window of infectivity'. The colonization percentages increased in all groups during the 6-year study period. The findings are thus in agreement with those of Straetemans et al. [1998]. Our study also demonstrates that a xylitol-associated reduction in the mother-child transmission of MS during the emergence of the primary teeth had a long-term influence on MS colonization. This finding is in accordance with studies in which CHX treatments of mothers have been used to reduce MS transmission [Köhler et al., 1983, 1984].

In the F group, the mothers were treated biannually with a fluoride varnish until the 2-year examination. Fluoride varnish treatments do not reduce MS levels in the oral cavity [Schaeken et al., 1991]. Thus, such treatment should have no effect on mother-child MS transmission. Although

the F group was relatively small, the colonization percentages at 3 and 6 years closely agree with figures published earlier for children of mothers with high salivary MS levels [Köhler and Andréen, 1994]. In the CHX group, the mothers' teeth were treated with CHX varnish at 6-month intervals until the 2-year examination. We found no differences in the maternal salivary MS levels among the three groups at any examination during the intervention [Söderling et al., 2000], or at the 3-year examination. At the 3-year examination, the children's colonization percentage in the CHX group was in between what we found in the xylitol and F groups, but, at the 6-year examination, the colonization percentages were similar in the CHX and F groups. The loss of subjects was similar in the xylitol and F groups but not in the CHX group, where all dropouts took place in the subgroup without MS colonization at the age of 2 years [Isokangas et al., 2000]. Due to the small sample size, no definite conclusions can be drawn from the results with CHX in the present study. According to the literature, a single application of the high-concentration CHX varnish used in our study reduces oral MS levels for several months [Schaeken et al., 1989]. A higher frequency of varnish treatments [Köhler et al., 1983] might have improved the results.

In conclusion, the xylitol-associated reduction in the probability of mother-child transmission of MS was still found in the children's MS counts at the age of 3 and 6 years.

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