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Xylitol Gum and Maternal Transmission of Mutans Streptococci

ABSTRACT

An important caries prevention strategy for children includes measures to interfere with transmission of mutans streptococci (MS). This study confirmed the effectiveness of maternal early exposure to xylitol chewing gum on mother-child transmission of MS. After screening, 107 pregnant women with high salivary MS were randomized into two groups: xylitol gum (Xylitol; $n = 56$) and no gum (Control; $n = 51$) groups. Maternal chewing started at the sixth month of pregnancy and terminated 13 months later in the Xylitol group. Outcome measures were the presence of MS in saliva or plaque of the children until age 24 months. The Xylitol-group children were significantly less likely to show MS colonization than Control-group children aged 9-24 months. The Control-group children acquired MS 8.8 months earlier than those in the Xylitol group, suggesting that maternal xylitol gum chewing in Japan shows beneficial effects similar to those demonstrated in Nordic countries.

KEY WORDS: xylitol, mother-child transmission, mutans streptococci, pregnancy.

INTRODUCTION

An important caries prevention strategy for children includes measures to impede the transmission of mutans streptococci (MS), consisting of *Streptococcus mutans* and *Streptococcus sobrinus*. Several reports have described that the earlier the MS colonization occurs, the greater the decay of children's teeth in later years (Köhler *et al*., 1988; Straetemans *et al.,* 1998; Isokangas *et al.,* 2000). Various interventions to prevent MS transmission in pregnant women and new mothers have been effective (Köhler and Andréen, 1994; Brambilla *et al*., 1998; Söderling *et al*., 2000; Thorild *et al.,* 2003).

Xylitol decreases the synthesis of insoluble extracellular polysaccharides *in vitro* (Söderling *et al.,* 1987). For that reason, habitual xylitol consumption might limit adhesion of MS to the enamel, thereby inhibiting MS transmission. In Nordic countries, mother-child studies xylitol studies in which intervention was started after delivery and terminated at the time close to or within the "window of infectivity" period for the children (Caufield *et al.,* 1993) produced similar results. Söderling *et al.* (2000) compared the effects of three strategies: biannual chlorhexidine (CHX) or fluoride varnish (F) treatments for mothers, or maternal xylitol gum (Xyl) chewing when the child was 3-24 mos old. When the children were 2 yrs of age, the MS prevalences were 9.7% (Xyl), 28.6% (CHX), and 48.5% (F). Thorild *et al*. (2003) reported that maternal xylitol gum chewing (child's age, 6-18 mos) significantly reduced MS transmission compared with chewing of the two control gums containing chlorhexidine/xylitol and sodium fluoride, with respective MS prevalences of 10%, 16%, and 28%.

This study was designed to confirm the effects of maternal chewing of xylitol gum at an earlier period (starting at the 6th mo of pregnancy and terminating when the child was 9 mos of age) than in previous studies of mother-child transmission of MS among the Japanese population. The hypothesis was that chewing xylitol gum would be better than basic prevention alone, which is generally implemented for pregnant women in Japan.

MATERIALS & METHODS

Participants

Pregnant women in the 3rd to 5th mos of pregnancy who visited the Miyake Obstetrics and Gynecology Clinic in central Okayama, Japan, were recruited for participation and interviewed with respect to their medical condition, specifically with respect to their prescription and over-the-counter medications history, and any gastrointestinal and TMJ problems. Individuals reporting antibiotic use during the prior month or GI problems were excluded. The salivary MS levels of the potential participants ($n = 400$) were estimated with the use of Dentocult SM Strip mutans

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(Orion Diagnostica, Espoo, Finland) according to the manufacturer's instructions. About half of the children of mothers with high MS counts show MS colonization at the age of 2 yrs (Berkowitz *et al*., 1981). Consequently, individuals with high MS counts, *i.e.,* Strip mutans scores ≥ 2 (equivalent to ≥ 10^5 CFU/mL) were sent an informational letter describing the study and inviting them to participate (n = 255). The letter included the phrase in Japanese, "We will offer you one of two oral hygiene programs: program-A (which gives priority to basic prevention) or program-B (chewing xylitol gum) to prevent cavity-causing bacteria transmission." Of those receiving the letter, 107 women (ages from 19 to 40 yrs; mean, 30.2) appeared and agreed to participate after providing fully informed consent to the study protocol. They completed baseline procedures, including a characteristics questionnaire. Reminder letters were sent repeatedly to non-responders, but reasons for non-response were not characterized further. The Okayama University Institutional Review Board approved this study (trial registration: http://www.umin.ac.jp/ctr/index/htm,UMIN000001690).

Study Design and Intervention

Each participant was assigned randomly to one of two groups—xylitol gum (Xylitol) or no gum (Control)—by a block randomization procedure, with the various block sizes unknown to the investigators. We used computer-generated random numbers to prepare the assignment schedule in advance. Assignment codes were implemented at Okayama University, remote from the study environment. The study was conducted at the Hello Dental Clinic, which was associated with the OB–GYN clinic. Both groups had equal basic prevention measures, including oral hygiene instructions and professional tooth cleaning. Xylitol gum was added to basic prevention measures so that we could examine whether those prior results were also true for Japan.

In the Control group, only basic prevention measures were given at the 6th mo of pregnancy. In the Xylitol group, maternal gum chewing was started in addition to basic prevention measures at the 6th mo of pregnancy and terminated 13 mos later, when the children were 9 mos old (Table 1). No child in either group experienced an intervention.

Table 1. Time Schedule of the Study

3–5 mos pregnant	Selection of pregnant women with high levels of salivary MS[a] (Strip mutans score ≥2)
6 mos pregnant	Intervention (IV) with xylitol chewing gum starts in Xyl[b] group. Oral examination (DMFT), oral hygiene instruction, and professional tooth cleaning were given to both groups.
9 mos pregnant (3 mos IV) delivery	
Child 6 mos (10 mos IV)	Saliva sample from tongue. Saliva sample from maxillary and mandibular ridges or plaque sample from tooth/child. Recording of the number of teeth for each child.
Child 9 mos (13 mos IV)	Saliva sample from tongue and plaque sample from tooth/child. Recording the number of teeth for each child. Intervention with xylitol chewing gum discontinues in Xyl group/mother.
Child 12 mos (3 mos after IV)	Saliva sample from tongue and plaque sample from tooth/child. Recording the number of teeth for each child.
Child 18 mos (9 mos after IV)	Saliva sample from tongue and plaque sample from tooth/child. Recording the number of teeth for each child.
Child 24 mos (15 mos after IV)	Saliva sample from tongue and plaque sample from tooth/child. Recording the number of teeth for each child.

[a] MS = mutans streptococci.
[b] Xyl = xylitol gum.
As incentives, both Xyl and Control (no-gum) groups were given two toothbrushes—for mother and child—at every visit. Implementation of Dentocult SM Strip mutans and its results were sent free of charge to each mother–child pair by mail until the child became 2 years old. Participants were asked to contact their family dentist for appropriate restorative treatment if treatment was required.

Sample Size

Based on the colonization rate described in the literature (Söderling *et al.,* 2000), 32 participants *per* group were needed to achieve a power of 90% to detect a difference of 35-40% (two-tailed, α = 0.05) (Browner *et al*., 1988). After adjustment for dropouts (30%), data for 46 participants *per* group were judged as necessary.

Gum and Adherence

Each gum pellet contained 1.32 g xylitol as the only sweetener, in addition to other ingredients: gum base, gum Arabic, calcium hydrogen phosphate, a *Gloiopeltis furcata* extract, flavoring, and glazing agents (XYLITOL®; Lotte Co. Ltd., Tokyo, Japan). Participants were instructed to chew 1 gum pellet for ≥ 5 min at least 4 times/day according to a review article showing 5-10 g xylitol/day chewed in increments ≥ 3 to be effective (Ly *et al*., 2008). Purchased by means of a research grant, the gum was given at each trimonthly visit during the intervention period as a three-month supply to promote and monitor compliance. On a provided Xylitol calendar, participants recorded the actual quantities of gum consumed daily and their abdominal symptoms: A, normal; B, loose stool; C, diarrhea; D, excessive gas; and E, constipation. Masticatory muscle fatigue and TMJ pain were also recorded when present.

Saliva and Plaque Sampling and Cultivation

Sampling from children was undertaken between 9:30 and 11:30 a.m. at 6, 9, 12, 18, and 24 mos of age during the study (Table 1). The mothers were instructed to refrain from giving food or drink or brushing the child's teeth for at least 1 hr prior to sampling.

For each child, samples were obtained from two sites: the tongue dorsum; and the mucosa of mandibular and maxillary ridges when a tooth was absent, or tooth surfaces when they were present. A strip was pressed gently against the child's tongue dorsum to collect an unstimulated saliva sample. A sterile cotton swab was used to collect the unstimulated saliva

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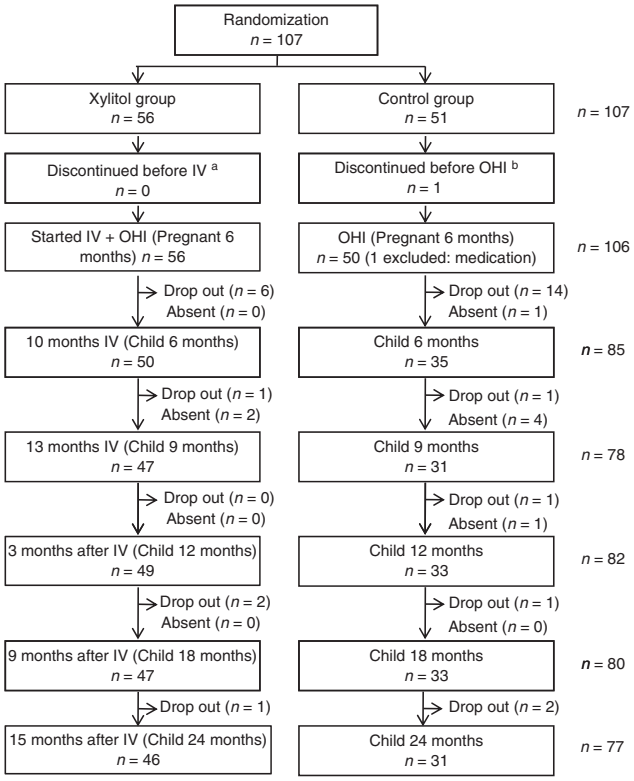

Figure. Trial profile during the 28-month study.
[a] IV = xylitol gum intervention.
[b] OHI = oral hygiene instruction and professional tooth cleaning.

sample by rotating it on mandibular and maxillary ridges, with immediate inoculation of it onto another strip surface when a tooth was absent. We used a micro-brush (Benda Microtwin; Centrix, Inc., Shelton, CT, USA) to collect a plaque sample from the buccal-cervical regions of tooth surfaces; dental floss was also used on all approximal surfaces of the erupted teeth. Such collection methods were validated previously (Karjalainen *et al.,* 2004). The plaque sample was then spread onto one of 4 roughened sites of the site strip (site 1, upper cervical; site 2, lower cervical; site 3, upper approximal; and site 4, lower approximal). The inoculated plaque site strip and the salivary strip were inserted into the culturing vial of Dentocult SM. Vials were transported to the university for incubation and assessment. In both the sampling and assessment procedures, the assignment was blinded.

After incubation at 37°C for 48 hrs, bacterial growth on the strips was assessed in comparison with the density chart by a single researcher whose intra-examiner reliability was $\kappa > 0.8$. For children, the following scores were used: 0 = not detectable; 1 = original scores 0 (detectable case) and 1; 2 and 3 = the same as the original, corresponding, respectively, to $< 10^4$, 10^4-10^5, 10^5-10^6, and $10^6 >$ CFU/mL (Jensen and Bratthall, 1989). The maximum score among the 4 sites was given as the representative score of each site strip.

The children were dichotomized as MS-positive (score 1-3 on either strip) or MS-negative (score 0 on both strips) for MS colonization.

Clinical Examination

The number of children's teeth was recorded at each examination. Maternal dental caries was examined clinically, in the 6th mo of pregnancy, by means of a sharp explorer and a mouth mirror with good illumination in a fully equipped dental chair. Then, after repeated calibration, two experienced dentists who were blind to the assignment recorded the measurements according to WHO criteria (1997). Inter-rater and intra-rater reliabilities of these examiners were high ($\kappa > 0.80$).

Statistical Procedures

The outcome measure was MS colonization in children. Using SPSS software (SPSS v. 15.0; SPSS Inc.), we performed chi-square and Fisher's exact tests for distribution of Strip mutans scores and prevalence of MS colonization between groups. Furthermore, we performed Kaplan-Meier survival analysis with the Mantel-Cox log-rank test to examine the age distribution of MS against the groups. We used Student's or Welch's *t* test, chi-square, and Fisher's exact tests to assess differences between groups. All *p*-values presented herein are two-tailed.

RESULTS

Initially, 56 participants were in the Xylitol group, with 51 in the Control group. At the study's end, the groups consisted of 77 mother-child pairs (46/Xylitol, 31/Control) (Fig.). The percentage of dropouts was 17% (10/56) in the Xylitol group and 39% (20/51) in the Control group. The primary reasons for participants' interruption were the family's change of residence and scheduling conflicts. No participant interrupted the study because of side-effects of the xylitol chewing gum. The mean frequency and dose of xylitol consumption were 2.9 times/day (range, 1.2 to 5.3) and 3.83 g/day, respectively, throughout the intervention period. The dropouts did not differ significantly from those who completed the study across groups with respect to the mother's age, the month of pregnancy, DMFT prior to intervention, child's birthweight, or gender distribution. The women who were invited and enrolled were somewhat older than non-enrolled women (30.2 *vs*. 28.8 yrs, $p = 0.02$), but no other differences were found.

The mean age (30.6/Xylitol *vs*. 29.9/Control yr, $p = 0.39$) and the mean DMFT (14.8/Xylitol *vs.* 14.9/Control, $p = 0.95$) of mothers at baseline, the mean birthweight of their children (2930/Xylitol *vs.* 2941/Control g, $p = 0.89$), and the children's gender distribution (%girl: 43.1/Xylitol *vs.* 41.2/Control, $p = 1.00$) were similar between groups. No significant difference between groups was found in the number of children's teeth that had erupted at any sampling time except for 12 mos of age (6.5/Xylitol *vs*. 8.2/Control, $p = 0.002$).

Effects on MS Acquisition and Transmission

Significantly more children in the Xylitol group exhibited non-detectable MS levels (score 0) on both the tongue and the gingival ridge or tooth surfaces at 9, 12, and 24 mos (Table 2). Plaque Strip mutans scores were likely to be higher than salivary Strip mutans scores. We combined the results from 2 sites to analyze the prevalence of MS colonization. Xylitol-group children were significantly

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Table 2. Distribution of Strip Mutans Scores for Each Sampling Site: Comparison Between Groups or Sites, and Prevalence of MS Colonization in the Infants Aged 6–24 Months in the Xylitol and the Control Groups

	6 mos (*n* = 85)		9 mos (*n* = 78)		12 mos (*n* = 82)		18 mos (*n* = 80)		24 mos (*n* = 77)	
Strip mutans scores[a] of two sites	Xylitol (*n* = 50)	Control (*n* = 35)	Xylitol (*n* = 47)	Control (*n* = 31)	Xylitol (*n* = 49)	Control (*n* = 33)	Xylitol (*n* = 47)	Control (*n* = 33)	Xylitol (*n* = 46)	Control (*n* = 31)
Saliva from tongue[b]										
Score 0	50 (100)[e]	34 (97.1)	46 (97.9)	24 (77.4)	44 (89.8)	18 (54.5)	37 (78.7)	19 (57.6)	33 (71.7)	12 (38.7)
Score 1	0 (0)	1 (2.9)	1 (2.1)	6 (19.4)	5 (10.2)	15 (45.5)	9 (19.1)	12 (36.4)	12 (26.1)	19 (61.3)
Score 2	0 (0)	0 (0)	0 (0)	1 (3.2)	0 (0)	0 (0)	1 (2.1)	2 (6.1)	1 (2.2)	0 (0)
Score 3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	$p = 0.412$[f]		$p = 0.014$*[g]		$p < 0.001$*[f]		$p = 0.121$[g]		$p = 0.007$*[g]	
Saliva from gingiva or plaque from tooth surfaces[c]										
Score 0	50 (100)	33 (94.3)	45 (95.7)	21 (67.7)	41 (83.7)	11 (33.3)	27 (57.4)	12 (36.4)	17 (37.0)	5 (16.1)
Score 1	0 (0)	2 (5.7)	2 (4.3)	9 (29.0)	7 (14.3)	17 (51.5)	17 (36.2)	17 (51.5)	20 (43.5)	22 (71.0)
Score 2	0 (0)	0 (0)	0 (0)	1 (3.2)	1 (2.0)	5 (15.2)	3 (6.4)	3 (9.1)	9 (19.6)	2 (6.5)
Score 3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.0)	0 (0)	2 (6.5)
	$p = 0.167$[f]		$p = 0.003$*[g]		$p < 0.001$*[g]		$p = 0.216$[g]		$p = 0.014$*[g]	
Strip mutans score difference between sites within groups	–	$p = 1.0$[f]	$p = 1.0$[f]	$p = 0.67$[g]	$p = 0.49$[g]	$p = 0.03$*[g]	$p = 0.08$[g]	$p = 0.30$[g]	$p = 0.001$*[g]	$p = 0.07$[g]
Prevalence of MS[d]										
MS -	50 (100)	32 (91.4)	45 (95.7)	18 (58.1)	41 (83.7)	9 (27.3)	26 (55.3)	9 (27.3)	17 (37.0)	4 (12.9)
MS +	0 (0)	3 (8.6)	2 (4.3)	13 (41.9)	8 (16.3)	24 (72.7)	21 (44.7)	24 (72.7)	29 (63.0)	27 (87.1)
	$p = 0.06$[f]		$p < 0.001$*[f]		$p < 0.001$*[f]		$p = 0.021$*[f]		$p = 0.035$*[f]	

[a] Strip mutans scores reflect MS levels given by Dentocult SM Strip mutans.
[b] MS levels in unstimulated saliva samples collected from the child's tongue.
[c] MS levels in unstimulated saliva samples from mucosa of mandibular and maxillary ridges when a tooth was absent, or in plaque samples from tooth surfaces when they were present.
[d] Results from two sites were combined to analyze the prevalence of MS colonization. The children were dichotomized as MS positive (score 1–3 on either strip) or MS negative (score 0 on both strips) for MS colonization.
[e] Figures are quantities of participants (percentages in parentheses).
[f] Fisher's exact test.
[g] Chi-square test.
*Statistically significant, $p < 0.05$.

less likely to be MS-positive than were Control-group children at and after 9 mos of age (Table 2). Survival analysis results suggested that Control group children acquired MS at a younger age than did Xylitol-group children (mean, 12.0 *vs.* 20.8 mos; median, 12.0 *vs.* 24.0 mos; log-rank test, $p < 0.001$). The children whose mothers did not chew xylitol gum acquired MS 8.8 mos earlier than did those whose mothers did chew the gum.

DISCUSSION

This report is the first to describe the effectiveness of maternal xylitol exposure during an earlier intervention period, starting at pregnancy and neither overlapping nor approaching the window of infectivity period for the children, in a non-Nordic population. The minimum intervention to achieve some effectiveness is useful from a public health viewpoint. Results of this study indicated that such intervention might prevent or delay mother-child MS transmission, corroborating such effects in this Japanese population, whose culture and dental public health services differ from those of Nordic countries, for which reports have been published.

The magnitudes of effects on interfering with transmission include the xylitol dose as well as the duration of intervention: 6-7 g/day for 21 mos (Söderling *et al.,* 2000) and 3.83 g/day on average for 13 mos (this study). Xylitol doses of 5-10 g/day have been effective for reducing both MS counts and caries occurrence (Ly *et al.,* 2008). The doses used in the Söderling study (2000) were within this limit. Those used in our study were close to the limit. Surprisingly, a very low xylitol dose, 1.95 g/day, has been reported to reduce MS transmission (Thorild *et al.,* 2003). A lower dose might inhibit MS production of insoluble polysaccharides. A higher dose might inhibit both MS production of polysaccharides and their growth, engendering reduction of MS counts.

We found that the prevalence of MS colonization in 24-month-old children of the Xylitol group was higher than that reported earlier for high-MS mothers (Söderling *et al.,* 2000; Thorild *et al.,* 2003). In those prior studies, the intervention periods were different. Dietary and some other habits might have differed among participants. Differences in sampling sites and methods might also have affected the results. One explanation is that differences in the distribution and prevalence of *S. mutans* and *S. sobrinus* might have

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influenced the infection rate. Studies conducted in several countries show that they vary (Tenovuo *et al.,* 1992; Milgrom *et al.,* 2000; Okada *et al*., 2002; Wu *et al.,* 2003; Yoo *et al*., 2007). Specifically, *S. sobrinus* has a significantly higher probability of transmission than *S. mutans*: the two species might have different acquisition properties (Kozai *et al.,* 1999).

This study had several limitations. One limitation was that incentives might have been insufficient to induce the compliance of the control group, as evidenced by its higher attrition rate. This study did not use a control gum, which might have affected blinding. However, the impact of chewing on mother-child transmission of MS would be negligible. Chewing a gum base 3-5 times a day (Söderling *et al*., 1997) or sugar-free gum sweetened with sorbitol/maltitol (Ly *et al*., 2008) did not decrease MS levels. Further studies are needed, however, to optimize this intervention method.

In summary, xylitol gum chewing, along with basic prevention measures with an early intervention period starting at pregnancy, significantly reduced mother-child transmission of MS in this Japanese population.

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