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Factors associated with mutans streptococci among young WIC-enrolled children

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Abstract

Objectives: The objective of this study is to assess relationships between the presence or absence of mutans streptococci (MS) and other covariates in children aged 12-49 months.
Methods: Data were analyzed using baseline information from 411 children enrolled in the Special Supplemental Food Program for Women, Infants and Children (WIC) who participated in a psychoeducational study in Iowa. Children were assessed for MS using a semiquantitative method (RODAC plates). Dental examinations using $d_1d_{2\text{-}3}$ criteria and presence of visible plaque on incisors and molars were completed. Mothers completed a series of detailed questionnaires regarding their child's oral health, their socioeconomic status, their child's dietary/oral hygiene habits, and beverage consumption, among other behaviors. Bivariate relationships with the presence of MS were assessed and followed by multivariable modeling using logistic regression (alpha = 0.05).
Results: MS was present in 144 children (35 percent). Bivariate relationships with MS presence were identified for multiple covariates that included demographic characteristics, type of beverage consumption, dental caries, and plaque measures. Multivariate logistic regression modeling suggested that MS presence in children was positively associated with nonwhite race of the child, number of teeth present, presence of cavitated lesions, proportion of teeth with plaque, and lower levels of maternal education.
Conclusions: The presence of MS was associated with greater caries and plaque scores and with low maternal education and nonwhite racial background.

Introduction

Early childhood caries (ECC) is an endemic disease disproportionately affecting very young children of low socioeconomic status (SES) and minority groups in the United States. ECC has a multifactorial etiology. Early mutans streptococci (MS) colonization, enamel hypoplasia, poor oral hygiene, and high frequency of sugar consumption are among many risk factors associated with ECC (1). If left untreated, ECC can cause pain and infection, affecting nutrition and other activities necessary for normal growth and development (2).

MS, mainly comprised of the species *Streptococcus mutans* and *Streptococcus sobrinus*, are the principal oral bacteria responsible for the initiation and development of dental caries in humans (3). MS can be transmitted from one individual to another. This transmission usually occurs vertically, from mother to infant. The relationship between maternal MS salivary levels and the risk of infant infection seems to be relatively strong as mothers with high levels of MS tend to have children with high levels, while those with low levels tend to have children with low levels (4). Mothers with high *S. mutans* levels who practice saliva-sharing activities such as sharing the same utensils with their children are reported to have the highest risk for transmitting *S. mutans* to their children (5). Horizontal *S. mutans* transmission, between members of a family or groups such as those found in day care, has also been reported in the literature but is generally not thought to be the primary mode of transmission in most populations (6).

The age at which MS is first acquired in children is thought to influence their susceptibility to caries; that is, the earlier the colonization, the higher the risk for future caries. Köhler *et al.* (7) collected saliva samples in 78 4-year-old children at 4-month intervals starting at 15 months of age and found that the earlier the MS had been detected in the children, the higher the subsequent caries experience. Approximately 90 percent of children colonized at 2 years of age had experienced caries and had a 10 times higher decayed and filled tooth surfaces (dfs) score at 4 years of age than children who did not carry *S. mutans* at this age. Alaluusua and Renkonen (8) reported similar findings in their study of 39 children. They concluded that early establishment of *S. mutans* not only affected caries prevalence but also to a great extent the levels of caries experience, as reflected in the number of new caries lesions during the study period. The proportion of MS in dental plaque and saliva samples has also been found to be high in children with ECC with up to 60 percent of total microorganisms being MS, compared with only 1 percent in children who are caries free (9). Lee *et al.* (10) also found a positive correlation between baseline percent of MS of total flora, cultured from maxillary incisors of 39 children aged 12-36 months and regrowth plaque scores at a 3-day interval. A positive correlation was also observed between baseline and regrowth plaque scores. Authors speculated that, at least in very young children, the presence of visual dental plaque on the maxillary incisors may be indicative of elevated MS levels.

The frequent consumption of beverages and foods containing sucrose increases the acidity of plaque and enhances the establishment and dominance of aciduric MS (11). One study of 1-year-old children found a strong correlation between nighttime consumption of sugar-containing beverages and total consumption of sugar-containing beverages with MS presence (12). Frequent sugar exposure, snacking, total sugar exposure, and sweetened fluids taken to bed were also shown to be associated with *S. mutans* colonization among 111 children followed from birth until 24 months of age (5). In a related study by the same authors looking at 6-month-old predentate infants, they found frequency of sugar exposure as the most important factor associated with *S. mutans* infection (13). In a study of 6- to 24-month-old children, authors found that children whose bottles contained sweetened beverages were four times more likely to be colonized by MS than children whose bottles contained milk (14). In a study of infants from birth to 18 months (15), the amount of MS was significantly correlated with the total number of eating/drinking events in a day and nearly significantly correlated with mean daily frequency of consumption of beverages and foods containing nonmilk extrinsic sugars. Furthermore, this study found that sex, social class, and mother's educational level were not associated with presence of MS; however, children who were not brushing by 12 months of age and with a habit of eating and drinking more than 6.7 times per day were more likely to be colonized by MS (15). A more recent study (16) examined the associations between diet and bacterial colonization among children aged 2-6 years. Results showed that *S. mutans*, *S. sobrinus*, and *Bifidobacteria* were all associated with the presence of severe ECC among the children. Authors also reported that children who tested positive to *S. mutans* had higher food cariogenicity scores.

The purpose of this study was to assess the relationship between the presence and absence of MS and children's dietary and oral hygiene habits as well as other covariates in high-caries risk children aged 12-49 months attending a Special Supplemental Food Program for Women, Infants and Children (WIC) in Iowa. WIC is a federally funded special supplemental nutrition program for women, infants, and children. At WIC, children receive a health screening by a certified nurse and a nutrition assessment by a licensed dietitian. To qualify for the WIC program, people must: a) live in the United States; b) have a nutritional risk such as inappropriate dietary habits; and c) have a limited income of 185 percent of the current federal poverty guidelines (17). Because the WIC program serves low-income populations, a great amount of dental disease occurs among the program's participants. Indeed, caries prevalence for WIC-enrolled children age 3 and under was reported to be 35-56 percent (18,19).

Methods

This study was approved by the University of Iowa Institutional Review Board and conducted analyses using baseline information of 411 healthy WIC-enrolled children 12-49 months of age who participated in a psychoeducational study in Iowa. Data were gathered from examinations of children, and the questionnaires were completed by mothers.

Dental examinations were conducted at two WIC clinic sites using the knee-to-knee technique by a single examiner (KWG). Dental plaque and caries status were recorded for all children following criteria described by Warren *et al.* (20). The number of maxillary/mandibular incisors and molars with visible plaque was inspected visually by the naked eye or with the aid of a dental explorer and without disclosing solution. The dental caries examinations using d_1d_{2-3} criteria, which distinguishes between cavitated and noncavitated carious lesions in the primary dentition, was completed using a DenLite® (Miltex, Inc., York, PA, USA) illuminated mirror and upon drying the teeth with gauze. When necessary, tooth brushing occurred prior to the dental examination in order to remove existing dental plaque. The examination was primarily visual, but an explorer was used to confirm areas of suspected decay.

Microbiological analysis was conducted semiquantitatively to determine MS levels present in the oral cavity of each of the study children. This analysis was performed by using a sterile wooden tongue blade and a RODAC™ (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) plate filled with a mitis salivarius agar base containing sorbitol, kanamycin sulphate, and bacitracin (MSKB agar). Similar microbiological analyses have been successfully reported in the literature as a valid method for assessing salivary MS levels (21). Unstimulated saliva was obtained by pressing a sterile wooden tongue blade against the dorsal surface of each subject's tongue 10 times (five times on each side of the tongue blade) to thoroughly coat it with saliva. Each side of the wooden tongue blade was then pressed onto the RODAC plate. The plates were kept chilled and transported to the University of Iowa laboratory and incubated in a CO_2 incubator for 48 hours at 37 °C. MS levels were then assessed by counting the number of colony forming units (CFUs), which resembled MS visible within the impression area. Because most of the children in our sample population ($n = 267$; 65 percent) had no MS CFU (Table 1), the levels of MS for the purpose of this study were dichotomized as absent versus present.

Mothers completed a series of questionnaires regarding their demographic background and their child's dietary habits, beverage intake, and other oral health behaviors such oral hygiene. Regarding beverage intake, mothers reported what beverage their child consumed during the week prior to their study appointment and indicated the number of servings the child drank and the amount drank per serving. Beverages were categorized as: a) milk-based beverages (milk, formula, and breastmilk), b) sugar-free beverages (diet soda pop, iced tea, and coffee), c) natural sugar beverages (100 percent juice), d) beverages with added sugar [juice drinks (Kool-Aid®, Kraft Foods, Northfield, IL, USA), sugared powder drinks, soda pop, sports drinks, and other sugared beverages], and e) all sugared beverages (combination of natural and added sugar beverages). Mothers also indicated what type of snacks and the number of times they were consumed by their children on a weekly basis. Carbohydrate-containing snacks were categorized based on the presence or absence of sugar and starch and the degree of processing of the starch (highly processed starches versus relatively unprocessed starches) as described in Marshall *et al.* (22) and categorized as either "cariogenic" or "non-cariogenic."

Table 1 Mother–Child Dyads' Sociodemographic Profile and Child's Clinical Variables*

Continuous-level variables	Mean ± (SD)	Range (median)
Mothers		
Mother's age (years)	27.6 (6.3)	17.4-51.6 (26.2)
Children		
Child's age (months)	27 (10.3)	12.2-49.4 (26.2)
Number of noncavitated lesions	1.69 (2.79)	0-27 (0)
Number of cavitated lesions	1.149 (2.06)	0-11 (0)
Number of teeth present	15.319 (5.05)	2-20 (16)
Number of molars and incisors with visible plaque	5.09 (4.61)	0-16 (4)
Proportion of molars and incisors with visible plaque†	0.36 (0.30)	0-1 (0.33)

Categorical-level variables	Frequency	Percent
Mothers		
Household income (>$15,001)	188	52
Household income		
$0-$5,000	90	25
$5,001-$10,000	46	13
$10,001-$15,000	40	11
$15,001-$20,000	80	22
$20,001-$25,000	41	11
$25,001-$30,000	25	7
$30,001-$35,000	24	6
$35,001-$40,000	15	4
$40,001+	3	1
Education (≥HS or diploma/GED)	331	81
Education		
Less than HS	16	4
Some HS	61	15
HS diploma/GED	251	62
2-year college degree	45	11
4-year college degree	20	5
Graduate degree	12	3
Children		
Race (nonwhite)	174	43
Race		
Caucasian	233	57
African American	65	16
Hispanic/Latino	53	13
Other race/ethnicity (including multi-racial children)	57	14
Sex (female)	207	51
Ever breastfed	210	51
Presence of mutans streptococci	144	35
Caries lesions		
No lesions	176	43
Noncavitated lesions only	66	16
Cavitated lesions only	18	4
Both noncavitated and cavitated lesions	151	37

* Not all participants responded to all questions.
† Proportion of molars and incisors with visible plaque was obtained by dividing the number of molars and incisors with visible plaque by the total number of molars and incisors present in the child's mouth.
HS, high school; GED, General Educational Development; SD, standard deviation.

Bivariate analyses and multivariable modeling were conducted to assess the relationships between presence of MS and other covariates. Bivariate analyses assessing the associations with covariates utilized Wilcoxon rank sum tests to assess the association of MS status with quantitative variables, chi-square and Fisher's exact tests for nominal categorical covariates, and Cochran–Mantel–Haenszel procedure using ridit scoring (23,24) for ordinal covariates. Logistic regression techniques were used to model the relationships of covariates to MS status. All tests used a significance level of 0.05.

Results

Table 1 shows the sociodemographic profile of participants. The mean age of the children was 27 months. Two-hundred thirty-three were white (57 percent) and 207 (51 percent) were female. MS was present in 144 children (35 percent). Forty-three percent ($n = 176$) of them were caries free, while 16 percent ($n = 66$) had noncavitated lesions only, 4 percent ($n = 18$) had cavitated lesions only, and 37 percent ($n = 151$) had both noncavitated and cavitated lesions. Mothers reported having breastfed 51 percent ($n = 210$) of children; only nine children (2 percent) were currently being breastfed. The mean age of mothers was 27.6 years. Twenty-five percent of the mothers lived in households earning $5,000 or less annually ($n = 90$), most were either married or lived with a significant other ($n = 205$; 50 percent) and had at least a high school diploma ($n = 331$; 81 percent) (Table 1).

Results of the bivariate analyses showed several statistically significant differences between the two groups of children defined by the presence or absence of MS in the mouth. In terms of demographics, the results indicated that children with MS present were significantly more likely to be nonwhite ($P < 0.001$) and to have mothers with less than a high school education ($P < 0.001$) (Table 2).

In considering associations with oral hygiene habits (Table 2), children with MS present in their mouth were more likely to use fluoridated toothpaste on a daily basis ($P < 0.001$). Such unexpected result may be explained by the fact that fluoridated toothpaste is more likely to be used regularly by older children, who in turn are more likely to have more teeth present and a longer period of time for MS colonization to occur when compared with younger children; this association was no longer significant after covariate adjustment in multivariable modeling. Having an adult participate in the daily brushing of the children's teeth was not statistically significant ($P = 0.058$) at the conventional 0.05 level, but may be considered suggestive, perhaps implying that parental help during the children's toothbrushing results in greater plaque removal and hence less MS. In addition, there was a negative association between a history of breastfeeding and the presence of MS ($P = 0.012$); this association was no longer significant after covariate adjustment in multivariable modeling.

A number of associations with beverage consumption were identified (Table 3). Children harboring MS consumed a higher percentage of sugared beverages of any type ($P = 0.001$). On the other hand, children not harboring MS were significantly more likely to drink a higher percentage of milk-based beverages ($P < 0.001$) on a daily basis. No difference was found between the two groups defined by MS status with regard to number of cariogenic snacks consumed daily ($P = 0.675$). However, an important fact to consider is that, as observed in Table 3, neither of the two groups consumed a high number of cariogenic snacks on a daily basis (mean = 1.15 for "MS Group" versus mean = 1.12 for "No MS Group").

Statistically significant differences between children with and without MS present were also found with respect to clinical variables studied (Tables 2 and 3). Children in the MS group were more likely to have cavitated lesions present (Table 2) and a higher number of noncavitated ($P < 0.001$) and cavitated lesions ($P < 0.001$) as well as a higher number ($P < 0.001$) and proportion ($P < 0.001$) of teeth with visible plaque (Table 3). Lastly, children with MS present were more likely to have more teeth present in their mouths ($P < 0.001$), reflecting both a greater number of surfaces exposed to risk and longer periods of exposure to risk (Table 3).

Table 4 shows the results of the final logistic regression model, which explored significant factors associated with the presence or absence of MS using forward stepwise logistic regression analyses. The results indicated that children with MS present were more likely to be nonwhite [odds ratio (OR) = 2.22, $P = 0.002$], to have cavitated lesions (OR = 3.40 $P < 0.001$), and to have mothers who had not earned a high school or general educational development diploma (OR = 2.75, $P < 0.001$). Furthermore, the presence of a greater number of teeth ($P = 0.007$) and a higher proportion of molars and incisors with visible plaque ($P < 0.001$) were also positively associated with a greater likelihood of harboring MS. No significant interactions or difficulties arising from multicollinearity were identified. One might take the view that caries status is further along the stream of causality; for this reason, we also modeled MS status without including caries status as a candidate predictor. The resulting model, arrived at by stepwise logistic regression, retained all of the other variables given in Table 4 and added no additional covariates. The ORs for these variables in the new model were very similar to those in the previous model.

As indicated in Table 3, there was strong evidence of bivariate associations with several of the beverage intake measures, identifying positive associations between the presence of MS and higher percentage consumption of added sugar beverages and all sugared beverages, and a negative association with the level of consumption of milk-based beverages. These associations were no longer significant in the context of multivariable logistic modeling (Table 4). Given that an important a *priori* goal of this research study was to examine possible associations

Table 2 Categorical Bivariate Associations with Mutans Streptococci

	Subgroup *n*	MS present (%)	*P* value†
Children			
Sex			0.213
Female	207	32	
Male	202	38	
Race/ethnicity			<0.001
White	234	25	
Nonwhite	177	49	
Race			<0.001
White	234	25	
African American	66	53	
Hispanic/Latino	51	45	
Other race/ethnicity (including multiracial children)	60	47	
Brush teeth daily			0.563
Yes	292	36	
No	113	33	
Brush teeth before bedtime			0.450
Yes	297	36	
No	88	32	
Use fluoride toothpaste			<0.001
Yes	231	44	
No	180	23	
Adult participates in brushing child's teeth			0.058
Yes	371	35	
No	11	63	
Ever breastfed			0.012
Yes	209	29	
No	202	41	
Caries lesions			<0.001
No lesions	176	15	
Noncavitated lesions only	66	26	
Cavitated lesions only	18	28	
Both noncavitated and cavitated lesions	151	64	
Any cavitated lesions*			<0.001
Yes	169	60	
No	242	18	
Mothers			
Household income			0.062
$0-$5,000	90	40	
$5,001-$10,000	46	35	
$10,001-$15,000	40	30	
$15,001-$20,000	80	38	
$20,001-$25,000	41	29	
$25,001-$30,000	25	36	
$30,001-$35,000	24	17	
$35,001-$40,000	15	20	
$40,001+	3	33	
Education			<0.001
<HS diploma/GED	80	60	
≥HS diploma/GED	331	29	
Education			<0.001
Less than HS	13	62	
Some HS	61	61	
HS diploma/GED	251	29	
2-year college degree	46	35	
4-year college degree	20	20	
Graduate degree	14	21	

* *Any cavitated lesions* includes all children with cavitated lesions, with or without noncavitated lesions.
† Significance probability associated with the test of association between MS status and the categorical variable of interest. Fisher's exact and chi-square tests were used to assess associations with nominal categorical variables, and ridit analysis was used to assess associations with ordinal-level variables.
HS, high school; GED, General Educational Development; MS, mutans streptococci.

Table 3 Bivariate Associations of Quantitative Measures with MS

	MS present		No MS present		
	n	% or mean ± (SD)	*N*	% or mean ± (SD)	*P* value**
Children					
Age (months)	144	32.24 (8.92)	267	24.26 (9.92)	<0.001
Daily beverage intake					
% Natural sugar beverage†	142	20.02 (14.88)	264	18.73 (14.10)	0.362
% Added sugar beverages‡	142	13.06 (17.43)	264	7.76 (12.78)	0.001
% All sugared beverages¶	142	33.07 (21.38)	264	26.49 (18.87)	0.001
% Milk-based beverages§	142	32.74 (20.48)	264	41.26 (20.28)	<0.001
Daily number of cariogenic snacks	144	1.15 (1.34)	264	1.12 (1.07)	0.675
Number of noncavitated lesions	144	3.28 (3.86)	267	0.83 (1.37)	<0.001
Number of cavitated lesions	144	2.44 (2.85)	267	0.42 (0.85)	<0.001
Number of molars and incisors with visible plaque	144	7.81 (4.60)	267	3.62 (3.91)	<0.001
Proportion of incisors and molars with plaque*	143	0.52 (0.29)	265	0.27 (0.28)	<0.001
Number of teeth present	144	18.18 (3.02)	267	13.77 (5.24)	<0.001

* Proportion of molars and incisors with visible plaque was obtained by dividing the number of molars and incisors with visible plaque by the total number of molars and incisors present in the child's mouth.
† Natural sugar beverage: 100% juice.
‡ Added sugar beverages: juice drinks (Kool-Aid), sugared powder drinks, soda pop, sports drinks, and other sugared beverages.
§ Milk-based beverages: milk, formula, and breastmilk.
¶ All sugared beverages: combination of natural and added sugar beverages.
** Significance probability associated with the Wilcoxon–Mann–Whitney test of the null hypothesis that the distribution of the quantitative outcome of interest is the same in the two groups based on MS status.
MS, mutans streptococci; SD, standard deviation.

between the presence of MS and dietary habits in young, low-income children considered to be at high risk for caries, analyses were undertaken to examine whether dietary measures were associated with those variables in the final model (Table 4). Findings, given in Table 5, show that the proportion of teeth with plaque was positively associated with the percentage intake of all sugared beverages ($P = 0.0001$) and negatively associated with the percentage intake of milk ($P < 0.0001$). As shown in Table 6, beverage intake profiles were seen to differ by education, race, and the presence of cavitated lesions. Children whose mothers did not have high school diploma or General Educational Development tended to have lower reported percentage consumption of milk-based beverages and greater percentage consumption of added sugar and all sugared beverages ($P < 0.0003$ in both instances). Beverage consumption profiles also differed significantly by race; notable results included higher percentage consumption of all sugared and added sugared beverages in nonwhite children ($P \leq 0.0001$) and lower consumption of milk-based beverage ($P = 0.005$). When caries status was considered, those children with cavitated lesions were reported to have lower percentage consumption of milk-based beverages ($P = 0.0001$) and higher percentage consumption of added sugar beverages ($P = 0.001$, Table 6).

Table 4 Logistic Regression Estimates for the Effects of the Independent Variables on the Presence of Mutans Streptococci (*n* = 411)

	Estimate (SD)	OR	95% OR, CI	*P* value
Race/ethnicity (nonwhite)	0.39 (0.13)	2.19	1.33, 3.61	0.002
<HS diploma/GED	0.51 (0.15)	2.78	1.52, 5.06	<0.001
Cavitated lesions*	0.60 (0.14)	3.33	1.92, 5.78	<0.001
Number of teeth present	0.11 (0.04)	1.12	1.03, 1.22	0.008
Proportion of molars and incisors with plaque†	1.48 (0.49)	4.04	1.68, 11.56	0.003

* *Cavitated lesions* includes all children with cavitated lesions, with or without noncavitated lesions.
† Proportion of molars and incisors with visible plaque was obtained by dividing the number of molars and incisors with visible plaque by the total number of molars and incisors present in the child's mouth.
OR, odds ratio; CI, confidence interval; HS, high school; GED, General Educational Development; SD, standard deviation.

Table 5 Relationship of Beverage Variables with Proportion of Molars and Incisors with Plaque

	Proportion of teeth with plaque		
	n	Spearman ρ	*P* value*
Total beverage intake	412	0.0400	0.418
% Natural sugar beverage†	407	–0.0045	0.929
% Added sugar beverages‡	407	0.2464	<0.0001
% All sugared beverages¶	407	0.1814	0.0002
% Milk-based beverages§	407	–0.2898	<0.0001

* Significance probability associated with the test of the null hypothesis that the Spearman rank correlation $\rho = 0$, i.e., that there is no increasing or decreasing relationship between the proportion of teeth with plaque and the specified variable.

† Natural sugar beverage: 100% juice.

‡ Added sugar beverages: juice drinks (Kool-Aid), sugared powder drinks, soda pop, sports drinks, and other sugared beverages.

§ Milk-based beverages: milk, formula, and breastmilk.

¶ All sugared beverages: combination of natural and added sugar beverages.

Discussion

The presence of MS was related to demographic, behavioral, beverage, and dental measures. As for demographics, data showed in both bivariate and multiple logistic regression analyses that nonwhite children and children of mothers with lower educational levels had a greater likelihood of having MS present (Tables 2 and 4). This finding was contrary to Habibian *et al.* (15), who reported SES, including mother's educational level, not to be associated with the presence of MS among 163 English infants. Although low SES has been reported to be a strong risk factor for bacterial colonization in children (25), few studies have examined this association. Our study found a weak association between the presence of MS and family income only approaching statistical significance at $P = 0.062$. Li *et al.* (25) found that lower family annual income was a significant predictor for early *S. mutans* colonization among 156 mother–infant pairs. The authors also reported that African–American mothers transmitted *S. mutans* with greater fidelity than white mothers; we cannot make any comparisons with this particular measure because our study did not assess bacterial transmission from mothers to their children.

We found that presence of MS was related to older children and higher number of teeth erupted in the child's mouth. Such finding is not surprising and is in accordance with Wan *et al.* (5). It is interesting to note that in the logistic regression analyses, child's age was not associated with MS presence once other variables were introduced into the model (Table 4). Whether this is a reflection of MS being established in a child's mouth at a young age or whether number of teeth, caries, and proportion of teeth with plaque were proxies for child's maturity cannot be adequately assessed in this cross-sectional analysis. Future studies using longitudinal data will be better equipped to answer this question. The number of any cavitated lesions was also strongly associated with the presence of MS at both bivariate and multivariate levels, confirming findings of other studies that reported high MS colonization levels associated with greater levels of caries among preschool-aged children (Tables 2 and 4) (9,26).

Bivariate analyses showed that having an adult participate in brushing the child's teeth approached significance ($P = 0.058$) with lower likelihood of MS presence (Table 2). Seow *et al.* (27) examined the effects of maternal dental health education/toothbrushing instruction on the levels of MS in 67 preschool-aged children and found that children who did not show MS infection at their first visit were those who reported greater frequency of toothbrushing. There were 26 children (29 percent) who converted from positive to negative results for MS infection between the first and second visits, which was attributed mainly to increased toothbrushing, as other dental health habits analyzed remained the same. The importance of toothbrushing in bacterial infection prevention among young children was also observed by Wan *et al.* (5) as parent-assisted toothbrushing was associated with noncolonization of *S. mutans* in dentate infants. The same was found by Habibian *et al.* (15), who reported that infants who had their teeth brushed by 12 months of age were less likely to have detectable MS in their plaque than those who had not initiated this practice. The proportion of molars and incisors with plaque in our study participating children was related to the presence of MS in both bivariate and multivariate analyses (Tables 3 and 4). Such finding is consistent to longitudinal studies that found that absence of oral hygiene and visible plaque were found to be major factors associated with bacterial colonization in young children (5,28). However, as pointed out by Lee *et al.* (10), people may experience higher levels of plaque not exclusively because of poor plaque removal and oral hygiene practices, but also as a result of biologic factors (i.e., colonization of specific bacteria). As discussed by the authors, "MS produce extracellular polysaccharides that may aid in dental plaque formation by adding to the mass of plaque and by strengthening the attachment of these adsorbed bacteria to the enamel (10)." Results of their study of children 12-36 months of age (10) showed a strong positive correlation between baseline and regrowth percent MS, which was minimally affected by the toothbrush prophylaxis done by the study examiner or the 3-day abstinence in oral hygiene measures. Nevertheless, educational messages for prevention of bacterial colonization and ECC should still focus on educating parents about their responsibility in establishing oral hygiene habits earlier in their child's life as well as brushing and effectively removing plaque from their child's teeth on a daily basis.

Results from the bivariate analyses in regard to dietary habits showed that children in our study who consumed more sugared beverages were more likely to be colonized by MS.

Table 6 Relationship of Beverage Variables with Education, Race and Presence of Cavitated Lesions

	Education												
	<HS diploma/GED						>HS diploma/GED						Wilcoxon
	n	Mean	STD	Median	IQR	Range	*n*	Mean	STD	Median	IQR	Range	*P* value*
Total intake	80	42.31	24.04	40.00	21, 58	0, 98	331	41.06	29.85	36.00	27, 48	0, 346	0.472
% Natural sugar†	79	20.48	16.16	18.92	8, 30	0, 65	327	18.87	13.92	16.67	11, 25	0, 100	0.511
% Added sugar‡	79	15.58	17.64	6.56	0, 29	0, 67	327	8.17	13.64	1.87	0, 12	0, 100	0.0003
% All sugared§	79	36.06	22.27	33.96	22, 50	0, 100	327	27.04	19.04	22.83	14, 35	0, 100	0.0003
% All milk-based¶	79	32.24	19.85	29.79	20, 40	0, 100	327	39.74	20.70	38.89	26, 55	0, 100	0.0008
	Race												
	Nonwhite						White						Wilcoxon
	n	Mean	STD	Median	IQR	Range	*n*	Mean	STD	Median	IQR	Range	*P* value
Total intake	177	40.74	35.06	35.43	22, 50	0, 346	234	41.73	22.99	38.00	29, 50	4, 206	0.087
% Natural sugar†	172	19.06	14.94	16.75	10, 26	0, 77	234	19.27	13.98	16.67	11, 25	0, 100	0.711
% Added sugar‡	172	14.23	17.62	7.14	0, 21	0, 100	234	6.22	11.14	1.08	0, 8	0, 70	<0.0001
% All sugared§	172	33.29	21.80	30.18	18, 48	0, 100	234	25.49	17.92	22.22	13, 33	0, 100	0.0001
% All milk-based¶	172	35.51	22.27	33.12	21, 50	0, 100	234	40.32	19.31	38.96	29, 54	0, 92	0.005
	Presence of cavitated lesions												
	Noncavitated lesions						Cavitated lesions						Wilcoxon
	n	Mean	STD	Median	IQR	Range	*n*	Mean	STD	Median	IQR	Range	*P* value
Total intake	260	43.63	31.82	38.29	29, 52	0, 346	151	37.30	22.16	33.71	21, 48	0, 144	0.012
% Natural sugar†	256	19.81	14.32	18.01	11, 25	0, 100	150	18.12	14.46	15.10	9, 26	0, 67	0.111
% Added sugar‡	256	8.12	13.78	1.43	0, 10	0, 70	150	12.15	16.06	6.23	0, 18	0, 100	0.001
% All sugared§	256	27.93	19.91	23.08	14, 36	0, 100	150	30.27	20.15	27.60	15, 40	0, 100	0.229
% All milk-based¶	256	41.00	20.75	41.69	27, 58	0, 92	150	33.63	19.91	32.29	22, 42	0, 100	0.0001

* Significance probability associated with the Wilcoxon–Mann–Whitney test of the null hypothesis that the distribution of % of total intake indicated was the same for the two specified groups. These results indicate that nonwhite children with poorly educated mothers are consuming more sugared beverages and less milk-based beverages. These beverage patterns are in turn associated with the presence of cavitated lesions.

† Natural sugar beverage: 100% juice.

‡ Added sugar beverages: juice drinks (Kool-Aid), sugared powder drinks, soda pop, sports drinks, and other sugared beverages.

§ All sugared beverages: combination of natural and added sugar beverages.

¶ Milk-based beverages: milk, formula, and breastmilk.

HS, high school; GED, General Educational Development; IQR, interquartile range.

Drinking more milk-based or less sugared beverages seemed to be protective against MS presence (Table 3). A strong correlation has been observed regarding high frequency of sugared beverage consumption and increased caries risk and experience (29,30). Consequently, an important preventive measure is to motivate parents to avoid giving their children sugared beverages, especially throughout the day, and to help them opt for other choices like milk or water for instance. Daily number of cariogenic snacks was not significantly associated with the presence of MS ($P = 0.675$) as it has been observed by Law and Seow (28) where they provided strong evidence that frequent consumption of sugar-containing snacks was associated with MS colonization in children 21-72 months old. However, as already pointed out, most of the study children were not consuming a high number of cariogenic snacks on a daily basis (Table 3). This low number of cariogenic snacks may not have been enough to place the study children at a higher risk for colonizing MS.

One of the primary research questions of our research team prior to starting the study data analyses was regarding the possible association between dietary habits and the presence of MS among a low-income high-caries risk young population. Data analyses showed that the variables related to the children's dietary habits (i.e., sugared beverage consumption) were significant only at the bivariate level (Table 3). These variables were not associated with MS presence in the logistic regression analyses once other variables were introduced into the model (Table 4). For this reason, our research team decided to further analyze which variables were correlated with the ones that entered the final model. Results showed that although dietary variables were not selected in the final logistic regression model, they were found to be significantly associated with a number of the covariates in the final model. For example, the proportion of teeth with plaque was positively associated with the percentage intake of all sugared beverages ($P = 0.0001$) and negatively associated with the percentage intake of milk ($P < 0.0001$) (Table 5). Maternal education was significantly associated with the percentage consumption of milk-based, added sugar, and all sugared beverages ($P < 0.0003$ in all instances). Interestingly, beverage consumption profiles differed significantly by race (Table 6). The numerous relationships among the variables provide a blueprint to discuss future action. Nonwhite children with poorly educated mothers are consuming more sugared beverages and less milk-based beverages. These are in turn associated with having cavitated lesions and a higher proportion of teeth with plaque. Such trend is observed in the scientific literature. Contemporary beverage consumption patterns among children suggest that milk and water intakes have declined, while the consumption of 100 percent juice and added sugar beverages have increased, resulting in numerous detrimental effects on children's overall health (31,32). The consumption of regular (nondiet) soda pop, regular powdered beverages and, to a lesser extent, 100 percent juice has been reported to be associated with increased caries risk (29). Differences in ethnic, educational, and socioeconomic levels have also been reported to influence dietary patterns (33-35). National data from WIC-enrolled children aged 1-5 years showed that Hispanic children consumed significantly more juice than non-Hispanic white children (36). Another national survey reported that 2- to 3-year-old African–American children consumed less milk products and more fruit beverages when compared with white and Hispanic children (37). The relationship between diet and education is presumed to influence people's food choices and understanding of health-related information. It is difficult to compare our results regarding the association between maternal education and children's beverage consumption with other studies because of the paucity of data on such association. However, studies regarding socioeconomic differences in food purchasing behavior, conducted in Australia, have shown that least educated shoppers were twice as likely to be less complaint with dietary guideline recommendations (33) and purchased fewer types of fresh fruit and vegetables when compared with their counterparts (34). Messages about the oral health dangers of sugared beverages and the benefits of milk-based beverages for children should be targeted to populations of poorly educated, minority families.

This study was subject to the inherent limitations of survey studies that rely on self-reported data collection. It is important to point out that this study targeted mothers of WIC-enrolled children in the state Iowa, and our sample population may not be entirely representative of other populations. Because this study employed a one-time saliva collection, it is possible that quantitation of salivary microorganisms on a single day and time did not adequately define numbers and proportions of cariogenic bacteria in these children. This is a limitation inherent in any cross-sectional analysis of cariogenic bacteria in children. However, we maintain that this one-time assessment provided an important snapshot of levels of cariogenic bacteria and more likely than not was representative of the load of cariogenic bacteria in the children in our study. Another important limitation is the fact that we did not collect salivary samples from the study mothers for MS analyses and did not collect information regarding their oral health status. However, it is important to note that one of the strengths of this study is our sample population that consisted of very young children from low-income families attending a WIC program. According to Kanellis (1), most children served by WIC should be considered at higher caries risk in general when compared with other children; therefore, preventive measures targeting these children are especially important. MS was found to be related to demographic, dental, and behavioral variables. Although we cannot determine temporal order and thus causality using cross-sectional data, evidence from these analyses present opportunities for future studies to investigate. Determining the factors associated with MS colonization is important in understanding risk factors associated with ECC as well as for implementing effective caries preventive measures targeted to children at increased caries risk.

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