Maternal Oral Mutans Streptococci (MS) Status, Not Breastfeeding, Predicts Pre dentate Infant Oral MS Status

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Abstract

The purpose of this study was to investigate infant oral mutans streptococci (MS) by feeding method and by the mother's oral MS status. For this cross-sectional data collection, full-term infants at 4–6 weeks of age and their mothers were tested for oral MS using the Dentocult® SM chairside test (Orion Diagnostica, Espoo, Finland). For the 104 mother–infant pairs (68 breastfed, 36 formula-fed), the two groups differed by mother's race/ethnicity ($p=0.006$) but not by delivery mode, level of care at birth, or MS in the mothers or the infants. Thirty-one percent of mothers and 12% of infants were MS positive. Infant MS was not predicted by mother's race/ethnicity, delivery mode, feeding method, or MS status. When stratified by feeding method, the mother's MS status did significantly predict the infant's MS status in the breastfed group (odds ratio=5.97; 95% confidence interval, 1.06–33.7; $p=0.043$). In conclusion, oral MS at $>10^4$ colony-forming units/mL were detected in 12% of the 4–6-week-old infants, and the effect of the mothers' oral MS status was modified by the feeding method. Future research is needed to clarify the modifiable perinatal and maternal risks associated with the development of the infant's pre dentate oral microbial environment, before the introduction of the first tooth.

Introduction

THE ROLE OF *Streptococcus mutans* and *Streptococcus sobrinus* (two closely related bacterial species, together referred to as mutans streptococci [MS]) in dental caries was first identified in 1924,¹ and in 1986, the often-observed link between oral *S. mutans* bacteria and dental caries was demonstrated.² Viable MS can be recovered from inanimate objects, such as a metal plate up to 24–48 hours after contamination, and MS is also transmittable directly through saliva.³ In the 1970s, Berkowitz et al.⁴ described the similarity of oral MS presence in mother and infant, and fidelity in terms of similarity of molecular fingerprint of *S. mutans* in the mother and her infant was demonstrated in the mid-1990s.⁵ Differentiation of *S. mutans* from *S. sobrinus* and positive detection for *S. mutans* were demonstrated using arbitrarily primed polymerase chain reaction fingerprinting in the late 1990s.^{6,7} In 1999 Kononen et al.⁸ showed that once oral anaerobes were established in the oral cavity as early as 2 months of age, they tended to persist. A review of published studies concerning sources of colonization of MS in children concluded that mothers appear to be the primary source,⁹ and breastfed infants were found at higher risk for oral MS compared with bottle-fed infants.^{10,11}

As an ancillary investigation within an ongoing clinical trial, we had the opportunity to examine oral MS status in 4–6-week-old pre dentate infants and their mothers. Therefore, the purpose of this study was to investigate the association of infant feeding method (breastfeeding versus formula feeding) with infant MS status, in the context of mother–infant pairs.

Materials and Methods

Ethical clearance for this study was obtained from the institutional review board at the Medical University of South Carolina. Mothers had granted informed consent in English or Spanish.

Data for this analysis were obtained from an ongoing prospective clinical trial of mother–infant pairs, in which all infants were more than 36 weeks of gestation at birth and the mothers had self-selected the feeding mode for their infant. The breastfed infants were allowed up to 10% formula usage, whereas the formula-fed infants were exclusively formula-fed. When the infant was 4–6 weeks old and before any intervention on the clinical trial, a chairside test for oral MS was performed on the mother and on the infant, using the Dentocult® SM Strip mutans test kit (Orion Diagnostica, Espoo, Finland) for detection of salivary MS. This kit was

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introduced in 1989 with reported sensitivity and specificity of 98% and 85%, respectively, and a positive predictive value of 93% and negative predictive value of 94%.¹² Oral sampling was performed from unstimulated saliva in both mother and infant. After incubation, the presence of colony-forming units (CFU) of MS per milliliter of saliva was scored from 0 to 3 according to the manufacturer. Negative status (score of 0) was indicated by $<10^4$ CFU/mL. Positivity for MS was indicated by a score of 1 ($\geq 10^4$ and $<10^5$ CFU/mL), 2 ($\geq 10^5$ and $\leq 10^6$ CFU/mL), or 3 ($>10^6$ CFU/mL). Inter- and intra-examiner agreement for three examiners reading the MS tests was 100%. Demographic and perinatal data for the mother and infant were abstracted from the parent study database. REDCap software was used for data entry. Tabulations, chi-squared test, Fisher's exact test (if any cell was less than 10), and logistic regression modeling were performed using STATA IC version 11.0.¹³

Results

From 111 mother-infant pairs who had MS status tested at the 4–6-week visit, complete data were available for 104 and are the subject of this analysis. Demographic data (Table 1) did not differ significantly if all 111 mother-infant pairs were included (data not shown). Among the 104 infants, 68 were breastfed, and 36 were formula-fed. Mother's race/ethnicity distribution was Hispanic (61%, $n=63$), white (22%, $n=23$), and African American (15%, $n=16$), and two pairs were classified as other (3%). The distribution of the mother's race/ethnicity differed significantly between the two feeding groups ($p=0.006$), with whites and Hispanics more likely to breastfeed and African Americans equally likely to breastfeed or use formula. However, the two feeding groups did not differ significantly by delivery mode (vaginal or cesarean),

by level of care at birth (newborn nursery versus higher level of care [e.g., Level II]), or by the presence of MS in the mothers or in the infants. Within each feeding group, the presence of MS in the infant's saliva did not vary significantly by mother's race/ethnicity (10–15% of infants were positive for MS in each ethnic/racial category; data not shown).

Overall, 31% ($n=32$) of the mothers and 12% ($n=12$) of the infants were positive for salivary MS (Table 2). In total, 69% (72 pairs) were congruent between mother and infant, and in 6% (six pairs) both mother and infant were MS positive. Infant oral MS status was not predicted by race of mother, by delivery mode, by feeding method, or by mother's salivary MS status (Table 3). However, when stratified by feeding method, the mother's salivary MS status did significantly predict the infant's salivary MS status in the breastfed group (odds ratio = 5.97; 95% confidence interval, 1.06–33.7; $p=0.043$) but not in the formula-fed group (odds ratio = 0.72; 95% confidence interval, 0.07–7.42; $p=0.782$). In multivariable logistic regression modeling this relationship was further modified by race of mother, but only among breastfed infants, where mother's MS status predicted the infant's MS status with odds ratio = 8.53, $p=0.027$ if race was included (data not shown).

Discussion

Our multi-ethnic study of 104 mother-infant pairs from coastal South Carolina found that 12% (12/104) of full-term infants were MS positive in saliva at 4–6 weeks of age using the Dentocult SM test. We found infants in both our breastfed (10%, 7/68) and formula-fed (14%, 5/36) groups were positive for MS at 4–6 weeks of age. We further found that salivary MS status of the infants was predicted by the mother's salivary MS status, but only if the infant was breastfed and not among formula-fed infants. That is, neither feeding method nor mother's salivary MS status alone was significantly associated with the infant's MS status. A comparable study found 9% (25/283) of Australian infants positive for MS at 5 weeks of age using the chairside diagnostic kit CRT[®] *S. mutans* test (Ivoclar, Melbourne, Australia).¹⁴ Positive maternal MS status ($>10^5$ CFU/mL), but not breastfeeding alone, was significantly associated with MS in the infant.¹¹

Using a plated culture technique for *S. mutans* detection, a relatively higher proportion of full-term Australian infants, 34% (44/128), was *S. mutans* positive at 11–15 weeks¹⁵ compared with our findings. Again, in a similar cohort of preterm infants, 60% (67/112), a higher proportion was positive for *S. mutans* at 6 months of age; the authors attributed the high proportions to their sample size and sensitivity of their culture media.¹⁰ Likewise, the proportions of preterm infants found to be *S. mutans* positive in the above studies are higher than in a recent study in a subset of 73 infants at 3 months of age from a small town of Northern Sweden,¹⁶ tested using the Human Oral Microbe Identification Microarray. Although *Streptococcus* Cluster III (which included *S. sobrinus*) was detected in $>90\%$ of samples, only 6.8% (5/73) of breastfed and 0.0% of formula-fed infants were positive for *S. mutans*.

In the above studies of preterm infants, there are differences in infant age (34 days to 15 weeks and then 6 months), geographic location (Australia, Sweden, and the

TABLE 1. CHARACTERISTICS OF MOTHERS AND INFANTS, BY FEEDING GROUP

	Breastfed (n = 68)	Formula-fed (n = 36)	Total (n = 104)	p value ^a
Race (mother)				
African American	8 (12%)	8 (22%)	16 (15%)	0.006
Hispanic	37 (54%)	26 (72%)	63 (61%)	
White	21 (31%)	2 (6%)	23 (22%)	
Other	2 (3%)	0	2 (2%)	
Delivery mode				
Vaginal	56 (82%)	27 (75%)	83 (80%)	0.602
Cesarean	12 (18%)	8 (22%)	20 (19%)	
No data	0	1 (3%)	1 (1%)	
Nursery				
Normal	63 (93%)	31 (86%)	94 (90%)	0.685
Level II	4 (6%)	3 (8%)	7 (7%)	
No data	1 (1%)	2 (6%)	3 (3%)	
Mother MS status				
Positive	23 (34%)	9 (25%)	32 (31%)	0.382
Negative	45 (66%)	27 (75%)	72 (69%)	
Infant MS status				
Positive	7 (10%)	5 (14%)	12 (12%)	0.748
Negative	61 (90%)	31 (86%)	92 (88%)	

^ap values exclude categories with missing data.
MS, mutans streptococci.

TABLE 2. ORAL MUTANS STREPTOCOCCI CONCENTRATION IN MOTHERS AND INFANTS

	Negative [$0 (<10^4)$]	Positive [score (CFU/mL)]			Total positive	Total
		1 ($\geq 10^4 < 10^5$)	2 ($\geq 10^5 \leq 10^6$)	3 ($> 10^6$)		
Mother	72 (69%)	19 (18%)	10 (10%)	3 (3%)	32 (31%)	104
Infant	92 (88%)	6 (6%)	6 (6%)	0	12 (12%)	104

Data are in colony-forming units (CFU)/mL.

United States), and most notably the methods for species detection and the grouping or not of specific species of the mutans group. For example, at one extreme, $1-10^3$ CFU/mL *S. mutans* was considered positive using the plated culture method,^{10,15} whereas both the Dentocult SM test and the CRT *S. mutans* test use a cutpoint of $>10^4$ CFU/mL for positive MS status. A recent study by Chaffee et al.¹⁷ used a plating and culture method for MS (*S. mutans* and *S. sobrinus*) and an a priori cutpoint of $\geq 4.5 \times 10^3$ CFU/mL for higher MS challenge. That cutpoint was based on an earlier study of Japanese mothers' whole saliva specimens for *S. mutans* and *S. sobrinus* that indicated that $\geq 4.5 \times 10^3$ CFU/mL was predictive of both MS colonization and prevalence of dental caries in children at 2.5 years of age.¹⁸ With our higher detection criterion of $>10^4$ CFU/mL, we likely underestimated the prevalence of MS in the saliva of both the mothers and infants, compared with using the cutpoint of $\geq 4.5 \times 10^3$ CFU/mL. Our estimate of the strength of the association of salivary MS in the mothers and the infants in the breastfed group is therefore also probably conservative.

In addition to the higher threshold for detection of salivary MS, a limitation of our study was the relatively small number ($n = 104$) of mother–infant pairs. This precluded further study

of the impact of perinatal variables (e.g., delivery mode). A delayed establishment of *S. mutans* in the vaginally delivered infants compared with infants delivered by cesarean section has been demonstrated.¹⁹ Also, within 5 minutes of birth, samples from neonates' skin, oral mucosa, and nasopharyngeal aspirate have been shown to closely resemble their own mother's vaginal microbiota for those vaginally delivered neonates, compared with resembling their mothers' skin surface microbiota found in the neonates delivered by cesarean section.²⁰

In summary, variables of microbial testing, including *S. mutans* and/or *S. sobrinus*, cutoff thresholds for CFUs, and age of infant at sampling, and perinatal variables, including delivery mode and feeding method, continue to challenge and change concepts of development of the oral microbial environment.

Conclusions

Our multi-ethnic study of 104 mother–infant pairs from coastal South Carolina found that 12% (12/104) of full-term infants were MS positive in saliva at 4–6 weeks of age using a cutpoint of $>10^4$ CFU/mL for positive MS status (Dentocult

TABLE 3. INFANT MUTANS STREPTOCOCCI STATUS AT 4–6 WEEKS OF AGE BY STUDY CHARACTERISTICS

	Infant MS status		Total (n = 104)	p value ^a	Odds ratio	95% confidence interval
	Negative (n = 92)	Positive (n = 12)				
Race (mother)						
Hispanic	55 (60%)	8 (67%)	63 (61%)	1.00	Reference	
African American	14 (15%)	2 (17%)	16 (15%)		0.81	0.16–4.18
White	21 (23%)	2 (17%)	23 (22%)		0.51	0.10–2.54
Other	2 (2%)	0	2 (2%)			
Feeding group						
Formula-fed	31 (34%)	5 (42%)	36 (35%)	0.748	Reference	
Breastfed	61 (66%)	7 (58%)	68 (65%)		0.71	0.21–2.43
Delivery mode						
Cesarean section	19 (21%)	1 (8%)	20 (19%)	0.322	Reference	
Vaginal delivery	72 (79%)	11 (92%)	83 (81%)		2.9	0.35–23.9
Maternal MS status						
Negative	66 (72%)	6 (50%)	72 (69%)	0.181	Reference	
Positive	26 (28%)	6 (50%)	32 (31%)		2.54	0.75–8.59
Breastfed group						
Mother MS negative	43 (70%)	2 (29%)	45 (66%)	0.039	Reference	
Mother MS positive	18 (30%)	5 (71%)	23 (34%)		5.97	1.06–33.68
Formula-fed group						
Mother MS negative	23 (74%)	4 (80%)	27 (75%)	1.000	Reference	
Mother MS positive	8 (26%)	1 (20%)	9 (25%)		0.72	0.07–7.42

^ap values exclude categories with missing data.

MS, mutans streptococci.

SM test). Development of detection methods for salivary MS that are easy to use and permit comparable units of CFUs is needed. Infant oral MS status was not solely predicted by race of mother, by delivery mode, by feeding method, or by mother's salivary MS status. However, when stratified by feeding method, the mother's salivary MS status did significantly predict the infant's salivary MS status in the breastfed group. Clarification of the contribution of perinatal factors related to the preterm infant's oral MS status and better understanding of the factors related to the infant's oral environment are needed to guide education and intervention activities.

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Disclosure Statement

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