

Familial Oral Microbial Imbalance and Dental Caries Occurrence in Their Children

Desequilibrio microbiano oral familiar y caries dental en los hijos

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DOSSIER CARIES DENTAL: INVESTIGACIÓN CLÍNICA Y EPIDEMIOLÓGICA

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ABSTRACT

Objective: Develop a familial liability index for oral microbial status that reflects an imbalance of oral domains based on the presence of risk indicators in saliva, inter-proximal plaque, tongue, and throat. **Methods:** Fifty-six mother-child pairs from Webster and Nicholas counties, West Virginia, USA, participated in this study. Saliva samples were assayed for *mutans streptococci* (MS), interproximal plaque samples for the BANA Test (BT) species, tongue swabs for BT, and throat swabs for any of the sentinel organisms (*Staphylococcus aureus*, *Streptococcus pyogenes*, and yeasts). The corresponding thresholds for a (+) risk indicator were, respectively, $\geq 10^5$ CFU of MS salivary levels, one or more BT-(+) plaques ($>10^5$ CFU/mg of plaque of at least one of BT-(+) species), weak-(+) BT for a tongue swab ($>10^4$ - $<10^5$), and $>10^4$ CFU/swab for any of the sentinel markers. **Results:** The mean age of mothers and children was 41.6 and 14.6 years. Ninety-one % of both mothers and children had at least one (+) risk indicator. Overall, 76% of mother child-pairs had at least one (+) concordant oral microbial risk indicator. Accordingly, the relative risk (RR) of children having concordant results with their mothers was increased 1.36 (BT-plaque), 1.37 (BT-tongue), 0.94 (sentinel organisms) and 1.13 (MS) times. Principal component analysis revealed distinct sets of oral microbial risk indicators in mothers and children that correlated with dental caries prevalence rates in children. **Conclusions:** Mother-child pairs shared similarities of oral microbial risk indicators that allow for the development of a liability index that can elucidate caries in the children.

KEY WORDS

Liability index, oral microbiology, mothers, children, dental caries.

THEMATIC FIELD

Cariology.

RESUMEN

Objetivo: Desarrollar un índice de responsabilidad familiar para el estado microbiano oral que refleje un imbalance de los dominios orales con base en la presencia de indicadores de riesgo en saliva, placa interproximal, lengua y garganta. **Métodos:** 56 parejas madre-hijo de los condados Webster y Nicholas, West Virginia, Estados Unidos, participaron en el estudio. Se analizaron muestras de saliva para *Streptococcus mutans* (Sm), placa interproximal y lengua (prueba de BANA), torundas de algodón para BANA, y garganta para organismos centinelas (*Staphylococcus aureus*, *Streptococcus pyogenes* y levaduras). Los umbrales correspondientes para un indicador de riesgo positivo fueron respectivamente $\geq 10^5$ UFC de niveles salivales de Sm, > 1 placas BANA positivas ($> 10^5$ UFC/mg de al menos una especie BANA positiva), BANA débil para torundas de lengua ($> 10^4$ - $< 10^5$), y $> 10^4$ UFC/torunda de cualquier marcador centinela. **Resultados:** Las edades promedio de madres e hijos fueron 41,6 y 14,6 años. El 91% madres y niños tenían al menos un indicador de riesgo positivo. El 76% de las parejas madre-hijo tenían al menos un indicador concordante de riesgo microbiano oral. Así mismo, el riesgo relativo de los niños con resultados concordantes con sus madres aumentó 1,36 (BANA-placa) 1,37 (BANA-lengua), 0,94 (organismos centinela) y 1,13 (Sm) veces. El análisis de componente principal reveló distintos grupos de indicadores de riesgo microbiano oral en madres e hijos que se correlacionaron con la prevalencia de caries en los hijos. **Conclusiones:** Las parejas madre-hijo compartieron indicadores de riesgo microbiano oral similares que permiten desarrollar un índice de responsabilidad que pueda dilucidar caries en los hijos.

PALABRAS CLAVE

Índice de responsabilidad, microbiología oral, madres, hijos, caries dental.

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Cariología.

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INTRODUCTION

The biodiversity and bioburden of oral microbial communities allow for the study of overall oral ecosystem imbalance and its relation to disease outcomes. The oral flora is composed of diverse, multi-species consortia composed of approximately 400 cultivable organisms in the subgingival domain (1) and over 1500 species in the supragingival domain (2) where many of these species have yet to be cultivated. The diversity and richness of the oral flora establishes itself within hours of birth presumably from the vaginal flora (3), health caregivers and from the environment. This heterogeneous oral ecosystem becomes more complex over the first two decades of life and is modulated by a variety of host, environmental, behavioral and genetic factors.

Saliva transmission often occurs in the lives of everyday families. Kissing, sneezing, yawning, sharing house utensils, the proximity and sometimes, the sharing of toothbrushes and other vehicles, make it possible to transmit and acquire viable bacterial species. There is evidence that the main source from which children acquire the mutans streptococci (4-6) is the mother. Most importantly, studies have demonstrated the possibility that decay, and the establishment of mutans streptococci in young children, can be reduced and/or prevented, by treating highly infected mothers prior to the eruption of the primary teeth in their offspring (7,8). Other studies (9-11) indicate that if the mutans streptococci do not colonize the primary teeth in the first year after their eruption, they are likely to remain caries free in subsequent years.

Transmission of periodontal pathogens such as *Porphyromonas gingivalis* is well documented in multi-generation families where contact with an infected member significantly increases the relative risk of colonization by *P. gingivalis* among intra-familial pairs (12). Studies looking at parent-child pairs with a rapid enzymatic test for the presence of *Treponema denticola*, *P. gingivalis*, and *Bacteroides forsythus* (BANA Test) have shown that children whose parents were colonized by BANA-positive species were 9.8 times more likely to be colonized by these BANA-positive species (13). Moreover, a study of mothers who had periodontal disease and their children (14) showed a remarkable similarity for bleeding upon probing and for BANA test results on six reference teeth of mother-child pairs.

The purpose of this study was to develop a liability signature for oral microbial status of mother-child

pairs that reflects a microbial imbalance of different oral domains (saliva, inter-proximal plaque, tongue and throat), and to relate it to dental caries prevalence rates in the children.

MATERIAL AND METHODS

This study was conducted in Webster and Nicholas Counties, West Virginia, USA. One hundred-three families were contacted by telephone from a pool of current attendants of the Camden-on-Gauley medical center at Webster County. The interview was conducted to invite participation of families with a child or children, ages 12 to 17, who were current patients of the Camden-on-Gauley Medical Center. One biological parent and child from each family was eligible to participate. Fifty-six mother-child pairs consented (the study protocol was approved by the University of Pittsburgh Institutional Review Board) and were enrolled into this study.

Demographic and Household Density Variables

The average age of mothers and children who participated in this investigation was 41.6 and 14.6 years, respectively. Mothers had on average 2.5 children living with them (range=1-5). Their household had an average of 3.5 bedrooms and a mean number of 1.6 bathrooms, which may have included a half-bath. Sixty percent of the children were females and 40% males.

Dental Caries Examination

Each child received a dental caries examination according to NIDCR criteria (15). Dental caries prevalence will be reported as surface-based caries prevalence rates (SBCPR) where the total number of carious surfaces was divided by the total number of surfaces (16). Similarly, surface-based restored prevalence rates (SBRPR) were computed for each child.

Determination of Mutans Streptococci Salivary Levels

Family members were given a paraffin pellet to chew it for one minute. Participants then swallowed the stimulated saliva. Two thirds of a specially treated plastic strip Dentocult SM (Orion Diagnostica, Espoo, Finland) was inserted into the mouth and rotated on the surface of the tongue about 10 times. This strip was placed into a culture vial containing a well-mixed bacitracin solution. The vial was incubated for 4 hours at 37 °C and results were scored on a scale of 0 to 3 (0-1: <100,000 mutans streptococci/ml of saliva; 2: >100,000 to <1,000,000 MS/ml; 3: >1,000,000 MS/ml).

A score ≥ 2 was the signature that a family member had a mutans streptococci infection.

Plaque Sampling of Putative Periodontal Pathogens and the BANA Test

The BANA test is a small plastic card to which two separate reagent matrices are attached, seen as strips on the card. The lower white reagent matrix is impregnated with N-benzoyl-DL-arginine-B-naphthylamide (BANA). Subgingival plaque samples are applied to the lower matrix, and then distilled water is applied to the upper matrix. The reaction occurs when the plastic strip is inserted into an incubator set at 55 °C for 5 minutes, at which temperature the highest sensitivity and specificity for the test can be achieved (17). By using a wooden toothpick (Stimudent), the toothpick was inserted into the interproximal space between the sampled teeth (4 inter-proximal sites between the distal surface of first molars and the mesial surface of second molars). Both sides of the toothpick were wiped onto the raised white reagent matrix on the lower portion of the test strip at the appropriate labeled location. There is room for four plaque samples on this strip. The BANA Test will show a blue color if positive when *T. denticola*, *P. gingivalis* or *T. forsythia* are present in the plaque samples. This indicates that one or more of the three BANA positive species are present at levels $> 100,000$ CFU. A weak-positive reaction (faint blue color) will indicate that one or more BANA-positive species are present at levels $> 10,000$ and $< 100,000$ CFU in the plaque sample. A negative reaction (no blue color) indicates that the BANA positive species are below the detection level of 10,000 CFU in the plaque sample. One or more BANA positive plaque samples conferred the signature of a family member being infected with one or more BANA-positive species.

Presence of Putative Periodontal Pathogens on Tongue Scraping Samples

Tongue scraping samples were obtained with the Stimudent wood toothpick. The sample was applied to the BANA test as described above. There is evidence that the tongue is a discrete oral site that can be heavily colonized by BANA-positive species (18) thereby constituting a source of intra- and extra-oral transmissibility of periodontal pathogens. A weak-positive BANA reaction for a tongue sample was the threshold of a family member being colonized with one or more BANA-positive species.

Assessment of Sentinel Organisms on Oropharyngeal Swabs

Throat swabs were collected by means of the Cultu-rete® swab system. In this system a throat swab is collected and placed in a tube containing transport media. These swabs were used to detect and quantify *Streptococcus pyogenes*, *Staphylococcus aureus*, and yeasts. These three sentinel markers were quantified in the BAP media. Obtaining throat swabs would emphasize clinical observations of oral infections potentially linked to an imbalance of the oral flora ecosystem attributable to poor oral hygiene and health related behaviors. A threshold of $>10,000$ CFU/swab for any of the sentinel organisms conferred the autograph of a family member being colonized with one or more of the oropharyngeal species, i.e., *S. pyogenes*, *S. aureus*, and yeasts.

Statistical Analysis

Concordance of oral microbial risk indicators between mother and child were estimated and corresponding relative risks were computed with the SAS statistical program version 9.2 (SAS Institute: Cary, NC, USA). Confirmatory factor analysis (CFA) was utilized to test unidimensionality of the four oral microbial indicators in mother-child pairs and of actual and past caries surface-based prevalence rates in the children. CFA analysis yields Principal Component outcomes (PC). The constructs developed given the signatures of the four microbial risk indicators and surface-based caries and restored prevalence rates allowed for the development of a CFA liability index specifying one latent variable as the source of co-variances between the constructs. This procedure, in addition to checking for unidimensionality, results in (a) the development of the intermediate liability index, and, (b) a second phase of data reduction, by selecting out the constructs having low factor loadings.

RESULTS

Table 1 shows the rate of oral microbial indicators in mothers and children. The prevalence of periodontal anaerobic infections was moderately high in both mothers and children where 64% of them exhibited at least one positive plaque sample for the BANA Test. BANA-positive species were also detected at moderate rates on the tongue of mothers and children confirming the notion that these species colonize the tongue surface. A high percentage of the children had at least one of the sentinel markers colonizing the pharynx. The rate of colonization of these organisms

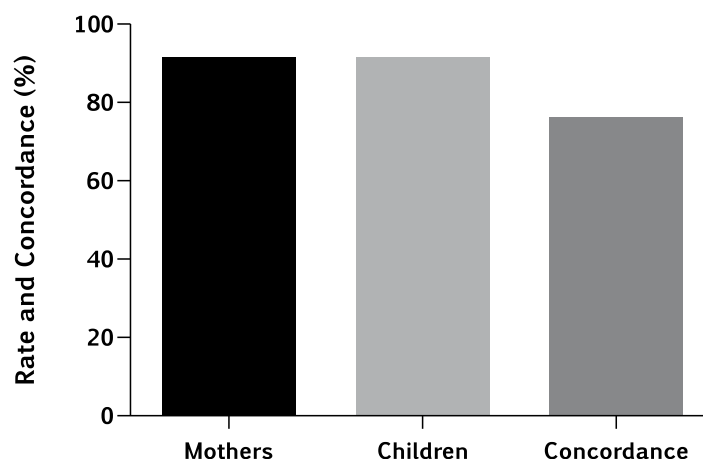
was moderate in mothers. Notably, mother-child pairs exhibited high levels of the mutans streptococci, i.e. $>10^5$ CFU/ml of saliva (Table 1). Children presented with an average surface-based caries prevalence rate of 1% and an average surface-based restored prevalence rate of 2.7%, for a total average of surface-based caries/restored prevalence rate of 3.7%.

TABLE 1
PREVALENCE OF ORAL MICROBIAL RISK INDICATORS
IN MOTHER-CHILD PAIRS

Indicator	Mothers	Children
Subjects with ≥ 1 BANA positive plaques	64% (of 48)	64% (of 55)
BANA weak-positive tongue-swab samples	35% (of 48)	49% (of 55)
subjects colonized by at least one sentinel organism $>10^4$ CFU/swab	41% (of 48)	60% (of 48)
Mutans streptococci score ≥ 2	65% (of 49)	54% (of 52)

Overall, 76% of mother child-pairs had at least one positive concordant oral microbial risk indicator (Figure 1). Ninety-one % of mothers and 91% of children had at least one positive oral microbial risk indicator. Thus, if a mother had at least one BANA positive plaque, her tongue colonized by BANA-positive species, at least one of the sentinel marker organisms, and high levels of the mutans streptococci, the relative risk of their children having concordant results was increased 1.36, 1.37, 0.94, and 1.13 times, respectively.

FIGURE 1
RATE OF OCCURRENCE OF AT LEAST ONE ORAL MICROBIAL RISK INDICATOR
IN MOTHERS AND CHILDREN. CONCORDANCE OF AT LEAST ONE ORAL MICROBIAL
RISK INDICATOR IN MOTHER-CHILD PAIRS



Additional analysis using principal component analysis allowed for the development of a liability index for oral microbial imbalance in mother-child pairs and the correlation of this imbalance with dental caries in the children.

The first principal component has variance (Eigen value) 1.98 and accounts for 20% of the total variance (Table 2). The results suggest that surface-based prevalence rates in children are highly correlated to a child having at least one BANA-positive plaque sample, a weak-positive BANA test for the tongue flora and high levels of the mutans streptococci. The second principal component has variance 1.65 and accounts for 16.5% of the total variance. This model suggests that an imbalance of the oral ecosystem in the mother (colonization of the tongue by BANA-positive species and presence of sentinel organisms on the pharynx) is highly correlated to surface-based caries prevalence rates in the children. The third principal component has variance of 1.56 and accounts for 15.5% of the variance. This model suggests that high salivary levels of the mutans streptococci in both mother and child and surface-based restored prevalence rates in the children are highly correlated.

TABLE 2
PRINCIPAL COMPONENT ANALYSIS OF DENTAL CARIES RATES AND ORAL
MICROBIAL RISK INDICATORS IN MOTHER-CHILD PAIRS

	PC1	PC2	PC3
Eigen value	1.98	1.65	1.56
% Variance	19.7	16.5	15.6
Correlation between variables and principal components			
Child surface-based caries prevalence rates	0.079	0.408	-0.107
Child surface-based restored prevalence rates	-0.294	-0.049	0.508
Mother ≥ 1 BANA-positive plaque(s)	-0.438	0.276	-0.373
Child ≥ 1 BANA-positive plaque(s)	0.633	0.463	-0.095
Mother BANA-weak (+) tongue flora	-0.176	0.678	0.296
Child BANA-weak (+) tongue flora	0.876	0.233	-0.073
Mother (+) sentinel organisms	-0.278	0.734	-0.054
Child (+) sentinel organisms	-0.203	-0.257	-0.637
Mother mutans streptococci levels >2	-0.302	0.034	0.675
Child mutans streptococci levels >2	0.533	-0.266	0.432

DISCUSSION

Our results allowed us to build a liability index for families based on the number of concordant oral microbial risk indicators present in the family which were subsequently confirmed by principal component analysis (Tables 1 and 2, Figure 1). Overall, 76% of mother child-pairs had at least one positive concordant oral microbial risk indicator (Figure 1). 91 % of both mothers and children had at least one of the oral microbial risk indicators. It is apparent that given the high level of infection by mutans streptococci and periodontal infections, and colonization of oropharyngeal sentinel markers in mothers and their children, that this child population could benefit from strategies based upon interfering with the acquisition of these organisms from mother to infant. Given the extensive documentation that mothers are the primary source of cariogenic and periodontal infections for

their children (4-6,19), then these children are an ideal population to design interventions to interfere with the transfer of mutans streptococci, periodontal bacteria and sentinel organisms from mothers to children, and possibly from other members of the family.

In that respect, early studies in Sweden have documented the potential for caries reduction by interfering with the transmission of the MS from mother to infant (7,8). The Swedish investigators have provided evidence that, if mothers who are highly infected with MS are aggressively treated so as to reduce their salivary MS levels, it results in a lower incidence of MS colonization in their children (7). The saliva of mother-infant pairs was periodically sampled for MS. When the children were 36 months old, 3 of 16 infants of the successfully treated mothers were infected (19%), versus 17 out of the 27 in the control group (63%). These findings show that the spread of MS to the children can be delayed, or prevented, by measures directed on the mother. But what was not initially suspected was that this delay would have long-term benefits on caries reduction. Thus, the children who were not initially colonized at age 2 had significantly less decay at age 4 than those children who harbored MS at age 2 (10). These long-term benefits were still present at the age of 7 as children who were colonized by the MS before 2 years of age had the highest caries experience and had higher levels of the MS when compared to those who were not colonized by the MS (20). Alaluusua and Renkonen reported a similar finding in Finland (9). In this study, infants demonstrably colonized with MS by age 2 had 30 times higher dfs scores at age 4 than children not demonstrably colonized with MS, i.e., an average dfs of 10.6 vs. 0.3, respectively. Recent studies in families of lower socioeconomic status have shown opposite results suggesting that suppression of oral infections in mothers did not translate into prevention of periodontal and cariogenic infections in their children (21). Our results in families of low socioeconomic status have clearly shown that infections by the MS in both mother and child were associated with surface-based restored prevalence rates and that high levels of infection by the MS in the child was associated with surface-based caries prevalence rates (Table 2).

Some organisms that inhabit the oral environment, particularly the pharynx, would be candidates to characterize an imbalance of the oral microbial ecosystem and reflect the biodiversity and uniqueness of this ecosystem. Consistent with this hypothesis we have shown that the detection of sentinel organisms in mothers was associated with dental caries rates in their children (Table 2)

Early studies of the BANA Test for the detection of *T. denticola*, *P. gingivalis* and *T. forsythia* demonstrated that 40% (n=20) of children whose parents had a documented history of periodontitis (as determined by evaluation of their dental records) had one or more BANA-positive plaques. Only 16% (n=137) of children whose parents' periodontal status was not documented had one or more BANA-positive periodontal plaques (22). These parents (n=157) were invited by phone to participate in a study where their periodontal status and the parent and child colonization by BANA positive species, was to be determined (13). Children whose parents had moderated to advanced periodontitis (n=20) were 12 times more likely to be colonized by BANA-positive species than children of periodontally healthy parents (n=14). These findings were further observed in a study of 28 periodontally diseased mothers and their 6 to 11 year old children (14). A remarkable similarity of periodontal clinical and microbiological parameters was noted. The mother-child pair was examined for bleeding upon probing and sampled for the BANA test on six reference teeth. A high agreement for bleeding upon probing on reference teeth was found for the mother child pair (74 to 96% concordance). A high agreement was also found for plaque samples of reference teeth that tested positive for the BANA Test in the mother-child pair (62 to 84% concordance). The similarity of bleeding upon probing between probands could reflect oral hygiene practices within the family. These data also demonstrated that BANA positive species are vertically transmitted between mother and their children. Notably, our results clearly show that periodontal infections in plaque and tongue samples in the child (a signature of imbalance of different oral domains) were associated with surface-based caries prevalence rates.

Overcrowded multigeneration dwellings, which are characteristic of families of lower socio-economic status as is the case in our cohort, may influence transmissibility of pathogens within households. Accordingly, family structure and household density may render persistence of oral microbial infections that are shared on a continuous basis, thereby adversely affecting the oral health of family members.

CONCLUSIONS

Mother-child pairs shared similarities of oral microbial risk indicators that allow for the development of a liability index that can elucidate caries in the children.

RECOMMENDATION

The development of a liability index for oral microbial imbalance in families may serve as a platform for studies of dental caries risk and associated-variables.

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