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Y. Lee[1], L.H. Straffon[1], K.B. Welch[2], and W.J. Loesche[3]*

[1]Department of Orthodontic and Pediatric Dentistry, School of Dentistry, University of Michigan, Ann Arbor, MI 48109-1078, USA; [2]Center for Statistical Consultation and Research, University of Michigan; and [3]Dept. of Biologic & Materials Sciences, Division of Prosthodontics, University of Michigan; *corresponding author, wloesche@umich.edu

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ABSTRACT

The oral microbial flora is unique, and available evidence indicates that it is passed vertically from parents to children. In this investigation, we used a chairside assay for the N-benzoyl-DL-arginine-2-naphthylamide (BANA)-sensitive enzyme found in *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythensis*, to determine the prevalence of these BANA-positive species in young children and their caregivers. We predicted that if the BANA enzyme was found in plaque samples of children, it would also be present in the plaque samples of the caregivers. Forty-four percent of 150 children had at least one plaque sample positive for the BANA enzyme. If the caregiver was BANA-positive, the odds of the child also being BANA-positive was 35 times more than for a child with a BANA-negative caregiver, after adjustment for the child's age and papillary bleeding score (PBS). Other significant predictors were the PBS of children ($p < 0.001$), a history of periodontal disease, and the ages of the caregivers ($p < 0.001$).

KEY WORDS: BANA test, children, transmission.

INTRODUCTION

Periodontal diseases are often neglected in childhood and adolescence. As evidence accumulates that adult forms of periodontal disease are opportunistic infections, due to certain indigenous bacterial species, there is interest as to when these periodontal pathogens colonize the oral surfaces. Some studies showed that if a child harbored a periodontal pathogen, then at least one of the parents will exhibit the same genotype of bacteria (Alaluusua *et al.*, 1991; Watson *et al.*, 1994). Kononen *et al.* (1992a,b) found that various anaerobic species colonize the edentulous mouths of infants, and that maternal saliva may act as a source of some Gram-negative anaerobes.

Tests capable of detecting various oral species have been developed, but DNA probes, immunological reagents, and bacterial culturing are laboratory-based and are not appropriate for chair-side use. Toward this end, there is need for a test that could be used reliably in routine clinical examinations to identify the presence of bacteria associated with periodontal diseases. The BANA (N-benzoyl-DL-arginine-2-naphthylamide) test detects a trypsin-like enzyme that is present in *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythensis*. The BANA test had 92% sensitivity and 70% specificity when compared with DNA probes and polyclonal immunological reagents (Loesche *et al.*, 1992).

Watson *et al.* (1991) showed that 56% of 157 children were positive for BANA-positive species in one or more of 4 samples of plaque, indicating that young children are colonized by *P. gingivalis*, *T. denticola*, and *T. forsythensis*. Children whose parents were colonized by BANA-positive bacteria were 9.8 times more likely to be colonized by BANA-positive species than were children whose parents were BANA-test-negative. Children whose parents had clinical evidence of periodontitis were 12 times more likely to be colonized by BANA-positive species. These data are compatible with the hypothesis that children may acquire the BANA-positive species from their parents, especially if the parent has periodontitis.

The purpose of our study was to obtain information regarding the prevalence of BANA-positive species in young children and their parents, by seeking evidence of the potential transmission of periodontal pathogens between parents and their children.

MATERIALS & METHODS

Subjects

Watson *et al.* (1991) found, with 34 caregivers, that it was possible to show a relationship between the presence of BANA-positive species in both the caregiver and the child. We sought to expand on that number by approaching caregivers who brought children to the pediatric clinic between September and December of 2003. After approval of the protocol by the University's Institutional Review Board, informed consent was obtained from 140 caregivers

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(107 mothers, 30 fathers, three grandparents) responsible for 150 children. The 150 subjects, ages 3 to 10 yrs, were seen in Graduate (n = 57) and Undergraduate clinics (n = 93) at the University of Michigan School of Dentistry. There were 86 boys and 64 girls, distributed in five racial groups: Caucasian (46.7%), African-American (33.3%), Asian (4.0%), Hispanic (13.3%), and others (2.7%).

Experimental Protocol

The parent who accompanied the child was asked to provide information about him/herself and the child, including birth date, gender, and age. Parents completed a questionnaire, with help provided if they had difficulties answering questions. The child was given a dental examination, including ascertainment of DMF score, caries status (caries-free, pit and fissure decay, moderate occlusal caries, proximal caries, rampant caries), and oral hygiene. Additional measurements were:

(1) A Plaque Index (PI) score was obtained at the interproximal site to be sampled for the BANA test (Barnes *et al.*, 1986).

(2) Interproximal/subgingival plaque samples were collected for the BANA test (OraTec@prodigy.net). A toothpick (STIM-U-DENT®, Johnson & Johnson, New Brunswick, NJ, USA) was used to obtain plaque samples in each quadrant. Any supragingival plaque was removed from the site, and a toothpick was inserted subgingivally between the first and second primary molars, if in the deciduous dentition; between the second primary molar and first permanent molar, if in the mixed dentition; and between the second premolar and first permanent molar, if in the late-mixed dentition or permanent dentition. If there were missing teeth, the plaque sample was removed from the mesial or distal side of the remaining tooth. The same procedure was repeated in caregivers.

(3) A Papillary Bleeding Score (PBS) was obtained. The PBS detects gingival inflammation and could be measured at the same time that the BANA sample was obtained (Loesche, 1979). After the Stim-u-Dent® was removed, any bleeding in the interproximal area was recorded on a 0 to 5 scale: 0, no bleeding, tissue healthy; 1, no bleeding, tissue not healthy; 2, spotting of blood; 3, bleeding with a flow in triangular fossa; 4, bleeding spreads to other teeth; and 5, spontaneous bleeding.

BANA Test

The toothpick with adherent plaque was wiped onto the lower strip of the BANA card. A separate toothpick was used for each plaque sample. After all tooth sites had been sampled, the upper strip was lightly moistened with distilled water by means of a cotton swab. The BANA card was folded at the perforation mark, so that the lower and upper strips met. The card was placed in an incubator at 55°C for 5 min. The BANA card was removed, and the lower portion was discarded in a manner appropriate for contaminated material. The color on the upper strip was recorded, by the consensus of two different examiners, as 'no blue' (negative), a 'faint blue' (weakly positive), or a 'distinct blue' (positive). The inter-examiner agreement was 96.7%. For statistical analysis, weakly positive and positive results were recorded as positive.

Statistical Analysis

SPSS 10.1 for Windows was used for statistical analysis. Frequency tables were generated for each categorical variable, and descriptive statistics were obtained for continuous variables. Chi-square tests were performed to assess the significance of the relationship of BANA test results to each of the categorical variables. Independent-samples *t* tests were performed to determine if there was a difference in the means of continuous variables for BANA-positive and BANA-negative patients. A stepwise logistic regression analysis was performed to determine the association between the various independent variables and the BANA-positive status of the child.

Table 1. Bacterial Colonization Based on BANA Test by Age and DMF Total (USA)

BANA-positive (sites)	n	%	Age Mean	Age (SD)	DMF (total) Mean	DMF (total) (SD)
0	84	(56%)	5.9	2.1	5.4	3.0
1	23	(15%)	6.7	1.9	4.2	2.6
2	28	(19%)	7.0	1.9	4.8	2.1
3	13	(9%)	7.5	2.2	5.0	1.9
4	2	(1%)	9.0	1.4	5.0	0.0

RESULTS

The 63 children with primary dentition had a mean dmft score of 6 (SD = 3.54). The 84 children with mixed dentition had a mean dmft score of 1.0 (SD = 1.47) and a mean DMFT score of 3.2 (SD = 2.04). The mean DMFT score for the three children with permanent dentition was 0.

Eighty-four (56%) of the children tested BANA-negative for all 4 plaque samples, and 66 (44%) tested positive and/or weakly positive in one or more of the samples (Table 1). Among the BANA-positive children, 23 had one positive sample, 28 had 2 positive samples, 13 had 3 positive samples, and two had 4 positive samples. Children with BANA-positive samples tended to have lower DMF scores compared with children with negative BANA samples, and those with a higher number of positive samples tended to be older (Table 1).

There was no significant relationship between the number of BANA-positive samples and gender, race, PI, caries status, the history of dental visits, brushing habits, or the antibiotic history of the subject ($p > 0.05$). Children who had a mixed dentition had significantly more BANA-positive samples than did children with a primary dentition ($p < 0.001$) (Table 2). Twenty-four percent of the children aged 3-5 yrs had BANA-positive samples, compared with 56.5% of the children aged 6 to 10 yrs ($p < 0.001$) (Table 2).

There was no significant difference between the mean dmft, DMFT, and total DMF (dmft+DMFT) for BANA-positive compared with BANA-negative children. The BANA-positive children were significantly older than the BANA-negative children. The BANA-positive children had a significantly higher PBS score (Table 2).

Seventy of the 140 caregivers tested BANA-positive and/or weakly positive in one or more of the quadrants. Eighty-four percent of children whose caregivers were BANA-positive were also BANA-positive, whereas only 7% of children whose caregivers were BANA-negative were BANA-positive ($p < 0.001$). Sixty-three percent of the children aged 3-5 yrs and 92% of the children aged 6-10 yrs, whose caregivers were

Table 2. Relationship of Clinical Variables to BANA Status

Variable	BANA Negative n (%)	BANA Positive n (%)	X^{2b}	df[c]	p-value
Treatment type					
Under TX	26 (47.3%)	29 (52.7%)			
Recall	37 (53.6%)	32 (46.4%)			
New patient	21 (80.8%)	5 (19.2%)	8.332	2	0.016
Dentition					
Primary	48 (76.2%)	15 (23.8%)			
Mixed	34 (40.5%)	50 (59.5%)			
Permanent	2 (66.7%)	1 (33.3%)	18.777	2	< 0.001
Age group					
3-5 yrs	44 (75.9%)	14 (24.1%)			
6-10 yrs	40 (43.5%)	52 (56.5%)	15.140	1	< 0.001
	Mean (SD)	Mean (SD)	*t*[a]	df	
dmft	4.7 (3.6)	3.9 (2.4)	1.64	144	0.103
DMFT	0.6 (1.3)	0.7 (1.2)	-0.67	148	0.504
DMF (total)	5.2 (3.4)	4.6 (2.4)	-3.6	147	0.184
Age	5.9 (2.1)	7.1 (1.9)	-3.64	143	< 0.001
PBS (mean)	0.2 (0.21)	0.8 (0.3)	13.42	110	< 0.001

[a] If no significant difference in variance was found, the pooled-variance *t* test is reported. If a significant difference in variance was found, the unpooled-variance *t* test is reported.
[b] X^2 = chi-square statistic.
[c] df = degrees of freedom.

BANA-positive, were also BANA-positive (Table 3).

If caregivers or family members had a history of periodontal disease, the children were significantly more likely to be BANA-positive ($p < 0.001$). Forty-seven children (62%) whose caregivers were ≥ 35 yrs old had BANA-positive scores, while only 26% of children who had younger caregivers had BANA-positive scores ($p < 0.001$).

We performed a stepwise logistic regression analysis to determine the association between various independent variables and BANA-positive results in the children. Each variable that was found to have a significant relationship to BANA-positive test results was included in the analysis. At each step, the most significant variables were entered into the model. At step one of the model, children with a BANA-positive caregiver had 69 times greater odds of a BANA-positive sample than did children with a BANA-negative caregiver. When the mean PBS and the age of the child were added to the model, they were also significant, and the model R^2 increased to 0.85 (Table 4). Other predictor variables—such as caregiver's age, family members with history of periodontal disease, occlusal development stage, and treatment status, which were significantly associated with the child's BANA-positive results in the bivariate models (Table 2)—were not significant.

DISCUSSION

The prevalence of aggressive (early onset) periodontitis in children in the United States is low (0.4-0.8%) (Löe *et al.*, 1991; Albandar and Tineco, 2002). These studies, based on radiographic assessments and detection of pocket depths, were unable to show the process of an ongoing inflammatory response to periodontopathic bacteria. Three bacteria associated with aggressive periodontitis—*P. gingivalis* (Lopez *et al.*, 1996), *T. denticola* (Albandar *et al.*, 1997; Mullally *et al.*, 2000), and *T. forsythensis* (Darby *et al.*, 2000)—are BANA-positive. Since the BANA test can be used at chairside to look for these species, this could be a means of detecting children at risk for, or actually experiencing, aggressive periodontitis.

The present study and that by Watson *et al.* (1991) showed that 44% and 56%, respectively, of the children had at least one BANA-positive plaque sample. BANA-positive plaque samples were more frequently encountered in older children with a mixed dentition, compared with younger children with a primary dentition. This could reflect that, with increasing age, there is more time for children's mouths to be colonized from outside sources. Another explanation could be that the micro-environment at interproximal sites in the mixed dentition, such as bleeding during eruption, promotes colonization of BANA-positive species from sites such as the tongue and the primary dentition. In this regard, the BANA species have nutrient requirements for host molecules, such as hemin, progesterone, ceruloplasmin, and acetyl muramic acid, among others (Loesche, 1968; Kornman and Loesche, 1982; Suzuki and Loesche, 1989). The eruption of the permanent teeth increases the number of interproximal sites that provide an ideal environment for anaerobic bacteria.

There was no correlation between BANA scores and PI or brushing habits. The mean value of the PBS was significantly correlated with the presence of BANA-positive plaques (Table 2), as has been found in adults (Kazor *et al.*, 1999).

The vertical transmission of periodontal microflora from mother to child has been reported (Könönen *et al.*, 1992b; Matto *et al.*, 1998). The present study found that children whose parents were colonized by BANA-positive bacteria were significantly more likely to have

Table 3. BANA Status of Caregiver *vs.* BANA Status of Child within Child's Age Category

Age Group			BANA (children) Negative	BANA (children) Positive	Total
3-5 yrs	BANA (caregiver)	Negative count (%)	33 (94.3%)	2 (5.7%)	35 (100.0%)
		Positive count (%)	7 (36.8%)	12 (63.2%)	19 (100.0%)
		Total count (%)	40 (74.1%)	14 (25.9%)	54 (100.0%)
6-10 yrs	BANA (caregive)	Negative count (%)	32 (91.4%)	3 (8.6%)	35 (100.0%)
		Positive count (%)	4 (7.8%)	47 (92.2%)	51 (100.0%)
		Total count (%)	36 (41.9%)	50 (58.1%)	86 (100.0%)

a positive BANA reaction. Also, children with BANA-positive plaques were more likely to have caregivers or family members who were older and had a history of periodontal disease ($P < 0.001$).

It is possible that a multi-factor risk model could be designed, like the early childhood caries risk model, to provide some benefit in the recognition and management of aggressive periodontitis. A limitation with the present concept(s) of the etiology of periodontal disease is the difficulty in explaining to young patients or parents why they are more or less at risk based solely on their level of oral hygiene. But an aggressive periodontitis risk model could be used to explain how an anaerobic infection could cause periodontal disease in either the child or the parent (Albandar, 2000; Albandar and Tineco, 2002).

An anaerobic periodontal infection risk model can be proposed from the present study. The BANA reaction of caregivers was the strongest predictor of BANA-positive plaque samples in the children. If the caregiver was BANA-positive, the odds of the child also being BANA-positive was 35 times more than for a child with a BANA-negative caregiver, after adjustment for the child's age and PBS. The R^2 of the model indicated that 85% of the variability was accounted for (Table 4). Use of this model could aid in identifying children who are at risk for periodontal disease and exclude those who are at low risk. A likely scenario would be to BANA-test children whose parents indicate a history of periodontal disease. If the child is BANA-positive, then his age and PBS could be used to estimate risk based on data provided in Table 4.

Table 4. Significant Predictors of BANA Status Based on Forward Stepwise Logistic Regression Analysis

n = 140	X^{2d}	df[e]	p-value	Odds Ratio	95%CI[f] Lower	Upper
Step 1[a]						
BANA caregiver	55.7	1	< 0.001	69.7[1]	22.9	212.6
Step 2[b]						
BANA caregiver	18.4	1	< 0.001	20.48[1]	5.1	81.4
PBS (mean)	17.8	1	< 0.001	5.032[2]	2.4	10.7
Step 3[c]						
BANA caregiver	17.3	1	< 0.001	35.29[1]	6.6	189.2
PBS (mean)	15.4	1	< 0.001	8.79[2]	1.2	26.0
Age	8.6	1	< 0.001	1.84[3]	1.2	2.8

a R^2 for model = 0.664.
b R^2 for model = 0.810.
c R^2 for model = 0.851.
d X^2 = chi-square statistic.
e df = degrees of freedom.
f CI = confidence interval.
1 Odds ratio for BANA-positive caregiver *vs.* BANA-negative caregiver.
2 Odds ratio for a 0.25-unit increase in mean PBS.
3 Odds ratio for a one-year increase in age.

Oral health risk assessment of this nature should focus efforts toward those children who have the greatest needs, thereby saving time and reducing expenditures. However, children in the present study were selected from patients with high caries scores and some behavioral issues. Therefore, a larger randomly selected population needs to be sampled for the results to be applicable to the general population. A long-term follow-up for those children who had the BANA reactions would be necessary to determine whether the risk indicators identified would predict which children are more likely to develop aggressive periodontitis. Longitudinal studies in adults showed that the presence of *P. gingivalis*, *T. forsythensis*, and BANA-positive plaque samples was a risk factor for periodontal disease (Beck *et al.*, 1990; Grossi *et al.*, 1994; Elter *et al.*, 1999).

An unexpected dividend from the present findings could be the observation that children who were BANA-positive always had a mother who was BANA-positive. Recent reports (Jeffcoat *et al.*, 2001; Lopez *et al.*, 2002) indicated a significant association between preterm and low-birthweight infants, and mothers with periodontal disease. Pediatric dentists who identify children with positive BANA results could ask the child's mother about her periodontal and pregnancy status. If the particular mother was pregnant, the dentist could recommend a periodontal examination.

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REFERENCES

Alaluusua S, Asikainen S, Lai CH (1991). Intrafamilial transmission of *Actinobacillus actinomycetemcomitans*. *J Periodontol* 62:207-210.

Albandar JM, Tinoco EM (2002). Global epidemiology of periodontal diseases in children and young persons. *Periodontol 2000* 29:153-176.

Albandar JM, Brown LJ, Löe H (1997). Putative periodontal pathogens in subgingival plaque of young adults with and without early-onset periodontitis. *J Periodontol* 68:973-981.

Barnes GP, Parker WA, Lyon TC, Fultz RP (1986). Indices used to evaluate signs, symptoms and etiologic factors associated with diseases of the periodontium. *J Periodontol* 57:643-651.

Beck JD, Koch GG, Rozier RG, Tudor GE (1990). Prevalence and risk indicators for periodontal attachment loss in a population of older community-dwelling blacks and whites. *J Periodontol* 61:521-528.

Darby IB, Hodge PJ, Riggio MP, Kinane DF (2000). Microbial comparison of smoker and non-smoker adult and early-onset periodontitis patients by polymerase chain reaction. *J Clin Periodontol* 27:417-424.

Elter JR, Beck JD, Slade GD, Offenbacher S (1999). Etiologic models for incident periodontal attachment loss in older adults. *J Clin Periodontol* 26:113-123.

Grossi SG, Zambon JJ, Ho AW, Koch G, Dunford RG, Machtei EE, *et al.* (1994). Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. *J Periodontol* 65:260-267.

Jeffcoat MK, Geurs NC, Reddy MS, Cliver SP, Goldenberg RL, Hauth JC (2001). Periodontal infection and preterm birth: results of a prospective study. *J Am Dent Assoc* 132:875-880.

Kazor C, Taylor GW, Loesche WJ (1999). The prevalence of BANA-hydrolyzing periodontopathic bacteria in smokers. *J Clin Periodontol* 26:814-821.

Kornman KS, Loesche WJ (1982). Effects of estradiol and progesterone on *Bacteroides melaninogenicus* and *Bacteroides gingivalis. Infect Immun* 35:256-263.

Könönen E, Asikainen S, Jousimies-Somer H (1992a). The early colonization of Gram-negative anaerobic bacteria in edentulous infants. *Oral Microbiol Immunol* 7:28-31.

Könönen E, Jousimies-Somer H, Asikainen S (1992b). Relationship between oral Gram-negative anaerobic bacteria in saliva of the mother and the colonization of her edentulous infant. *Oral Microbiol Immunol* 7:273-276.

Löe H, Brown LJ (1991). Early onset periodontitis in the United States of America. *J Periodontol* 62:608-616.

Loesche WJ (1968). Importance of nutrition in gingival crevice microbial ecology. *Periodontics* 6:245-249.

Loesche WJ (1979). Clinical and microbiological aspects of chemotherapeutic agents used according to the specific plaque hypothesis. *J Dent Res* 58:2404-2412.

Loesche WJ, Lopatin DE, Stoll J, van Poperin N, Hujoel PP (1992). Comparison of various detection methods for periodontopathic bacteria: can culture be considered the primary reference standard? *J Clin Microbiol* 30:418-426.

Lopez NJ, Mellado JC, Leighton GX (1996). Occurrence of *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis* and *Prevotella intermedia* in juvenile periodontitis. *J Clin Periodontol* 23:101-105.

Lopez NJ, Smith PC, Gutierrez J (2002). Higher risk of preterm birth and low birth weight in women with periodontal disease. *J Dent Res* 81:58-63.

Matto J, Saarela M, Alaluusua S, Oja V, Jousimies-Somer H, Asikainen S (1998). Detection of *Porphyromonas gingivalis* from saliva by PCR by using a simple sample-processing method. *J Clin Microbiol* 36:157-160.

Mullally BH, Dace B, Shelburne CE, Wolff LF, Coulter WA (2000). Prevalence of periodontal pathogens in localized and generalized forms of early-onset periodontitis. *J Periodontal Res* 35:232-241.

Suzuki M, Loesche WJ (1989). Ceruloplasmin can substitute for rabbit serum in stimulating the growth of *Treponema denticola*. *Infect Immun* 57:643-644.

Watson MR, Lopatin DE, Bretz WA, Ertel IJ, Loesche WJ (1991). Detection of two anaerobic periodontopathogens in children by means of the BANA and ELISA assays. *J Dent Res* 70:1052-1056.

Watson MR, Bretz WA, Loesche WJ (1994). Presence of *Treponema denticola* and *Porphyromonas gingivalis* in children correlated with periodontal disease of their parents. *J Dent Res* 73:1636-1640.