

Influence of Age on the Reactivity of the BANA Test Among Brazilian Children

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Three hundred and twenty samples of subgingival plaque were obtained from 80 caucasian girls, ranging from 10 to 13 years of age. The samples were analyzed to verify the influence of age upon colonization of the gingival sulcus by microorganisms potentially pathogenic to the periodontal tissues.

The gingival and plaque status were evaluated through the gingival index (GI) and plaque index (PII) and the microflora was assessed by the enzymatic method benzoyl-arginine-naphthylamide (BANA).

The results of the BANA test were positive for 62.50% of the tested individuals and 40% of the examined sites. The influence of age was statistically significant on BANA reactivity, and the number of positive sites was greater at 11 (57.5%) than at 12 years (28.8%).

Key Words: BANA test, spirochetes, children.

Introduction

Studies on the development of periodontal disease in childhood have demonstrated that, despite the presence of bacterial plaque, the disease appears less frequently in the primary dentition phase than during adulthood. This fact may be attributed to host inborn resistance and lack of response, especially in this age range (Mackler and Crawford, 1973). Other possible factors are a difference in inflammatory response (Longhurst et al., 1977; Matsson, 1978) and in the extent of chertinization, and greater tissue vascularization (Longhurst et al., 1977).

On the other hand, it should be pointed out that a possible modification of the microflora with advancing age (Longhurst et al., 1977; Matsson and Goldberg, 1985; Waite and Furniss, 1988), as well as increased host response (Waite and Furniss, 1988) may contribute to the increased tendency towards the development of gingivitis.

The subgingival microflora of children is known to differ from that of adults, with some components possibly being absent or reduced in number (Bailit et al., 1964; Araújo and McDonald, 1964; Socransky and Manganiello, 1971; Delaney et al., 1986). Among these components are spirochetes, and in particular *Treponema denticola*, which, in addition to the pathogenicity mechanisms detected in this genus, has the ability to synthesize a trypsin-like enzyme that acts on the synthetic substrate benzoyl- arginine-naphthylamide (BANA), and whose detection demonstrates the presence of this microorganism in the subgingival microflora.

According to Loesche et al. (1987) and Bretz and Loesche (1987), the BANA test could be used to identify active sites of periodontal disease and to determine the urgency of treatment, as well as the need for treatment monitoring and for retreatment.

As observed in adults, the BANA test was found to be able to identify the samples of subgingival plaque of children that are capable of hydrolyzing the BANA substrate (Watson et al., 1989, 1990; Fonseca, 1990), which means that, even when periodontal disease is not yet clinically detectable or presents unclear signs, these sites can be considered to be at risk for development of the disease.

Thus, the objective of the present study was to determine the influence of age on the detection of some important periodontal pathogens in samples of subgingival microflora from 10- to 13-year old girls using the BANA test.

Material and Methods

The study was conducted on 80 Caucasian girls aged 10 to 13 years, selected from public elementary schools in the city of Araraquara, State of São Paulo. Children with dental braces and children with obvious signs of systemic disorders or regularly using any medication were excluded from the study.

Clinical procedure

The selected children were examined for the presence of plaque and for gingival health status using the plaque index (PII) and the gingival index (GI) according to the criteria of Löe (1967). The indices were applied a maximum of 1 week before the study by a single examiner with experience in their use who utilized a probe with millimeter marks and a buccal mirror, both duly sterilized, and who was aided by an assistant trained in data recording procedures.

For the collection of subgingival microflora, the buccal cavity was divided into quadrants, and 4 sites related to the presence or absence of bleeding were selected on the basis of the GI data.

The supragingival plaque was removed with gauze and discarded and the subgingival plaque was removed with the aid of a sterilized wooden wedge (Inodon) held with sterilized hemostatic pliers.

The wedge was introduced into the sulcus with gentle movements and without pressure for removal of plaque samples which were immediately placed aseptically in coded test tubes containing 0.6 ml reduced transport fluid (RTF) prepared according to Syed and Loesche (1972).

Bacteriological procedure

Four to five sterilized glass beads were placed in each of the test tubes containing the plaque samples and the tubes were shaken in a Mixtron-Toptronix mixer at maximum speed for 20 seconds (Loesche et al., 1987) for microorganism dispersal.

After dispersal, a 0.05 ml aliquot of each sample was removed with an automatic micropipette (Camelo) fitted with disposable tips and deposited into each well of a special U-bottomed hemagglutination plate (Microtit) commonly used for immunological reactions. RTF (0.05 ml) was placed in two wells as reaction control. A 0.1 ml aliquot of BANA solution prepared according to Bretz and Loesche (1987) was then added to each sample-containing well. The plates were carefully wrapped with plastic film (Magipak) to prevent evaporation of the material and incubated at 37°C for 17 to 24 h, according to the method of Loesche et al. (1987).

After incubation, the plastic film was removed and a 0.025 ml aliquot (1 drop) of 10% sodium dodecyl sulfate, (CH₃)₂ SO (Merck), was added to each well, followed by the addition of a 0.025 ml aliquot (1 drop) of Fast Garnet. The plate was again wrapped with plastic film and incubated in an oven at 37°C.

Readings were taken by an examiner and an observer, the first one always being taken after the addition of Fast Garnet. New readings were taken after 30 min, 1 h and 18 h and comparative notations were made (Bretz and Loesche, 1987).

The results were scored with numbers corresponding to the colors observed, according to the method of Loesche et al. (1987) as follows: 1, yellow = negative; 2, orange = weakly positive; 3, pale red = positive; 4, dark red = strongly positive.

Statistical analysis

For data analysis, the GI and PII values obtained were grouped into bleeding and non-bleeding gingiva and visible and invisible plaque, respectively, according to Bordoni et al. (1982), Matsson and Goldberg (1985), Mikx et al. (1986) and Van Oosten et al. (1988).

Thus, on the basis of the criteria for application of their different degrees, GI values of 0 to 1 were considered to be the non-bleeding group, and GI values of 2 and 3, the bleeding group. Similarly, PII scores of 0 and 1 were considered to be invisible plaque, and scores of 2 and 3 as visible plaque. In an analogous manner, the results of the BANA test were considered to be: 1 = negative, and 2, 3 and 4 = positive.

Data were analyzed using the GLIM 3.77 (Generalized Linear Interactive Modeling) program, with the level of significance set at 5% in the chi-square distribution.

Results

The results are presented in Tables 1 and 2. Analysis of the data showed that the hypothesis that age does not affect BANA reactivity should be rejected since the chi-square value obtained (15.273) was significant (5 d.f., $p < 0.01$). The results showed that 50 (62.5%) of 80 children examined were BANA-positive (Table 1). However, when sites are considered, only 128 (40.0%) of a total of 320 presented a positive reaction. Table 2 shows that the highest frequency of BANA-positive reactions (57.5%) occurred at the age of 11 years, and the lowest (28.8%) at the age of 12.

Table 1 - Distribution, frequency and percentage of BANA-positive sites among 80 girls, according to age group.

Age	Patients per group (m)	BANA-positive children (n)	BANA-positive sites (100 n/ 50) %	Age (100 n/ m) %
10	20	11	22.0	55.0
11	20	16	32.0	80.0
12	20	9	18.0	45.0
13	20	14	28.0	70.0

Table 2 - Number and percentage of BANA-positive and BANA-negative sites in 80 children, according to age.

BANA	Age (years)							
	10		11		12		13	
	No.	%	No.	%	No.	%	No.	%
Negative	50	62.5	34	42.5	57	71.2	51	63.8
Positive	30	37.5	46	57.5	23	28.8	29	36.2

Discussion

The need for auxiliary clinical means for the determination of active sites of periodontal disease, for treatment monitoring and for the identification of periodontal pathogens at sites refractory to conventional treatment has led to a large number of studies based on dark field and phase contrast microscopy and on the search for simplified biochemical methods.

According to Loesche (1986), the presence of the trypsin-like enzyme capable of hydrolyzing the synthetic substrate of trypsin (BANA) and produced by pathogens such as *B. gingivalis*, *T. denticola*, *B. forsythus* and a species of the genus *Capnocytophaga* (Laughon et al., 1982) could be used as a diagnostic marker. The BANA test reveals the presence of this enzyme through a chromogenic reaction obtained by adding one drop of Fast Garnet to the suspension (Laughon et al., 1982; Loesche, 1986).

According to Loesche (1986), BANA hydrolysis reflects the presence of spirochetes in the subgingival plaque in relation to the other microorganisms cited above. The BANA test can also be used as an indicator of morbidity since ≥ 7 mm deep pockets showed the highest percentage of positive reactions.

In addition to its use as an indicator of active periodontal disease, the BANA test could be used as a means of monitoring treatment, with the possibility of predicting future episodes of periodontal injury (Loesche et al., 1987; Bretz and Loesche, 1987).

The possibility of making a prognosis about sites likely to develop periodontal disease was confirmed by Loesche et al. (1990) in a study in which they compared the BANA test in the form of a card (Perioscan) and the ELISA in the detection of *T. denticola* and *B. gingivalis*. The authors concluded that the precision was comparable in 84% of the samples and that both tests detected periodontopathic microorganisms in the absence of clinical disease, although the BANA test had the advantage of being faster, more sensitive and less expensive.

The demonstration that the BANA test is a reliable indicator of the presence of increased proportions of spirochetes, and of *T. denticola* in particular, and has the potential for use as an indicator of periodontal disease (Syed et al., 1984; Loesche, 1988; Schmidt et al., 1988; Loesche et al., 1990) justified its use in the present study in which girls aged 10 to 13 years were evaluated in an attempt to clarify the increased inflammatory response of the gingiva during puberty.

The present results demonstrated a positive BANA reaction in 50 (62.50%) of the 80 children studied. The distribution of BANA-positive individuals as a function of age demonstrated a greater percentage at 11 years (80.0%), with a lower percentage at 12 years (45.0%), reaching 70% at 13 years of age (Table 1). When the test is considered in relation to the total of 320 sites studied, 40.0% of them were positive.

These results are similar to those obtained by Fonseca (1990), who, using the same methodology, detected 45.08% BANA-positive sites and 78.78% BANA-positive children in a study carried out on 5- to 10-year old children. The present data are also comparable to those reported by Watson et al. (1989) who, in a study using the BANA test in the form of a card (Perioscreen), observed at least one BANA-positive or weakly positive site in 59.0% of the children examined.

These data confirm early colonization of the subgingival environment by some periodontal pathogens, and *T. denticola* in particular, so that these sites can be considered

at risk in terms of the development or exacerbation of periodontal disease, as proposed by Loesche et al. (1987), Bretz and Loesche (1987), and Schmidt et al. (1988).

Colonization of the gingival sulcus of children by spirochetes occurs during early childhood, as suggested by Moore et al. (1984), Braun et al. (1986) and Loesche (1988) and confirmed by Fonseca (1990) when they detected their presence at 27.50% of the sites studied in ten 5-year old children. This possibility was also confirmed by Mikx et al. (1986), who detected a high percentage of sites with spirochetes among children aged 7 to 9 years.

In the present study, correlation of positive sites with age demonstrated that age significantly affects BANA reactivity. Table 2 shows that positivity to the test increased from 10 (37.5%) to 11 (57.5%) years, decreased at 12 years reaching the lowest percentage (28.8%), only to increase again at 13 years of age (36.2%). Thus, positive reactions increased with age, a trend previously observed in the study by Fonseca (1990).

The higher positivity observed at 11 years of age is justified by the possible effect of female sex hormones, which start to be secreted during puberty, on the subgingival microflora. At increased levels or in a situation of disequilibrium, these hormones may cause modifications of the microflora with a consequent increase in certain pathogens (Kornman and Loesche, 1980; Delaney et al., 1986; Van Oosten et al., 1988; Mombelli et al., 1989).

On this basis, essential conditions for the growth of certain periodontal pathogens can be reached through given elements that replace growth requirements (in this case, female sex hormones) or through the possible increase in the number of sites of greatest depth encountered in mixed dentition (Fonseca, 1990), together with the change in the inflammatory response of tissue observed with increasing age.

On the other hand, the lower frequency of positive reactions detected at 12 years despite the permanence of the hormonal factor are explained by the statement of Parfitt (1957) that this age is usually related to better oral hygiene habits, probably due to the girls' awareness of the importance of their appearance.

Colonization by different pathogenic microorganisms, spirochetes among them, during the late phases of adolescence (Wojcicki et al., 1987) may justify the increased positivity observed in 13-year old girls.

Thus, the pattern of increased percentage of BANA-positive tests with age may be justified by the possible increase in the number of certain microorganisms as ideal growth conditions favor the transformation of the microflora into a more pathogenic one. In addition, the tissue response to bacterial plaque, which increases slowly from childhood to adulthood because of changes either in the cell infiltrate or in the vascular response (Longhurst et al., 1977; Matsson, 1978; Matsson and Goldberg, 1985), may lead to the increase of certain pathogens.

In view of the fact that the inflammatory state may influence the composition of the microflora and that hormonal factors may also have an effect, it is necessary to evaluate the effect of these variables on the reactivity to the BANA test during puberty by using a parameter of physiological maturation.

Conclusions

1. The BANA test was positive in 62.50% of the girls studied and in 40.0% of the sites examined.
2. The highest percentage of sites with a BANA-positive reaction occurred at 11 years (57.5%) and the lowest (28.8%) at 12 years.
3. Age had a significant influence on the reactivity to the BANA test.

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