BANA Test in Subgingival Plaque and Levels of Gingival Inflammation in Brazilian Children

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The benzoyl-DL-arginine-naphthylamide (BANA) enzymatic test was used as an alternative diagnostic method in the study of the levels of gingival inflammation (non-bleeding and bleeding sites) of 66 Brazilian children of both sexes aged 5 to 10 years. The BANA data showed that of the 240 sites studied, 89 non-bleeding sites (43.63%) and 30 bleeding sites (50.00%) were BANA positive, whereas 115 non-bleeding sites (56.37%) and 30 bleeding sites (50.00%) were BANA negative. However, when the BANA-positive data were correlated and submitted to statistical analysis they did not show statistical significance (P > 0.05).

Key Words: subgingival plaque, children, BANA test.

Introduction

Several studies have shown the existence of many children with gingival and periodontal diseases, changes or problems that are not diagnosed by routine examination, possibly causing premature loss of deciduous teeth or damage to supporting tissue during adulthood.

The considerations about the factors that probably influence the etiology of periodontal disease during childhood and prepuberty are based on epidemiologic, experimental and clinical investigations that almost invariably demonstrate a relationship between plaque levels and levels of periodontal disease (Lightner et al., 1971; Spencer et al., 1983). However, current research has focused on the study of the subgingival microbiota using very simple to highly sophisticated techniques and very modern and advanced technological resources such as culture, isolation, immunology, DNA probes, microscopy, and enzyme research, among others, in addition to epidemiologic, experimental and clinical investigations.
Thus, using enzyme assays, Laughon et al. (1982) and Loesche (1988) determined that *T. denticola*, *B. gingivalis*, *B. forsythus* and *Capnocytophaga* produce a trypsin-like enzyme that can be visualized by hydrolysis of the synthetic substrate benzoyl-DL-arginine-naphthylamide (BANA). On this basis, using a test card (Perioscreen), Watson et al. (1990) demonstrated that the BANA test can detect periodontopathic microorganisms in children and also suggested that the presence of these bacteria may be related to the presence of the same bacteria in their parents.

Fonseca et al. (1993), using the BANA test on the subgingival plaque of 66 Brazilian children aged 5 to 10 years, observed that the test was positive in 78.8% of the children and in 45.0% of the sites examined.

Orrico et al. (1991), in a study of the interaction between BANA test, gingival status and age in 10-13-year old children, noted a significant effect of age, with the highest risk of positive reactivity occurring at 11 years in the presence of bleeding gingiva (74.1%), a value twice that observed at 12 years in the presence of bleeding gingiva (33.3%). The authors concluded that there was a statistically significant effect of gingival status on the reactivity to the test.

Thus, the objective of the present study was to carry out the BANA test on the subgingival plaque of Brazilian children as a function of the presence or absence of gingival inflammation.

Material and Methods

The test was applied to 264 subgingival plaque samples from 66 randomly selected children of both sexes aged 5 to 10 years, enrolled at the State Elementary School "Antonio J. de Carvalho" and at the SESI-339 Educational Center of Araraquara, SP.

Children wearing dental braces, or taking any medication or presenting obvious signs of systemic disorders were excluded from the study. The children included in the sample were submitted to determination of the gingival index (GI) according to the criteria of Löe (1967). The index was determined by a single examiner and by a previously trained annotator, using a probe with millimeter divisions and a buccal mirror, both duly sterilized.

For the collection of subgingival bacterial plaque, the buccal cavity was divided into quadrants and, considering the GI data, 4 sites (1 per quadrant) were selected according to the presence or absence of bleeding.

Supragingival plaque was removed with gauze and discarded and the subgingival plaque was removed with the aid of a sterilized wooden wedge (Indon Ind. Ed. Exp. Imp. de Produtos Odontológicos Ltda.) held with a sterilized hemostatic forceps as recommended by Fonseca et al. (1993) and immediately placed under aseptic conditions into 13 x 100 mm test tubes containing 0.6 ml reduced transport fluid (RTF) prepared by the method of Syed and Loesche (1972), for the maintenance of viability of anaerobic microorganisms.

The BANA test was carried out as recommended by Loesche et al. (1987) and Bretz and Loesche (1987) and processed by the method of Fonseca et al. (1993).
Data were analyzed statistically using the GLIM 3.77 software (Generalized Linear Interactive Modelling). For the processing of the statistical analysis, the scores recommended by Loesche et al. (1987), used for the reading of color intensity, were grouped as recommended by Schmidt et al. (1988), Loesche et al. (1990) and Fonseca et al. (1993).

To facilitate data analysis, we used the criteria of bleeding and non-bleeding areas, grouping the 0 and 1 and the 2 and 3 scores of the GI criteria of Löe (1967), which differ precisely in terms of the absence or presence of gingival bleeding upon gentle probing (Ainamo and Bay, 1975; Matsson and Goldberg, 1985).

Results

The results of the BANA test for samples from bleeding and non-bleeding sites showed that of the 264 sites examined, 89 (43.63%) non-bleeding sites and 30 (50.0%) bleeding sites were BANA positive, whereas 115 (56.3%) non-bleeding sites and 30 (50.0%) bleeding sites were BANA negative.

Analysis of these results showed the absence of a significant association between these variables ($\chi^2 = 0.758$; d.f. = 1; $P < 0.05$), i.e., there was no statistical evidence that the occurrence of a positive BANA test differed in the presence or absence of bleeding gingivitis.

On the basis of the positive and negative BANA tests and of non-bleeding and bleeding sites, an attempt was made to add the age factor.

Table 1 shows the number of sites submitted to the BANA test, as well as data about gingivitis and age. These data were not analyzed statistically because an expected value of less than 5 was often presented. It is interesting to note, however, that the proportion of BANA-negative tests for bleeding sites was greater than the proportion of BANA-positive tests for bleeding sites at the ages of 6 and 7 years, very close for the ages of 5 to 8 years and lower for the ages of 9 and 10 years.

<table>
<thead>
<tr>
<th>BANA</th>
<th>Gingivitis</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Negative</td>
<td>Non-bleeding</td>
<td>23</td>
<td>57.5</td>
<td>21</td>
<td>47.72</td>
<td>21</td>
<td>43.75</td>
</tr>
<tr>
<td></td>
<td>Bleeding</td>
<td>3</td>
<td>7.50</td>
<td>6</td>
<td>13.63</td>
<td>8</td>
<td>16.66</td>
</tr>
<tr>
<td>Positive</td>
<td>Non-bleeding</td>
<td>12</td>
<td>30.00</td>
<td>14</td>
<td>31.81</td>
<td>17</td>
<td>35.41</td>
</tr>
<tr>
<td></td>
<td>Bleeding</td>
<td>2</td>
<td>5.00</td>
<td>3</td>
<td>6.81</td>
<td>2</td>
<td>4.16</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>40</td>
<td>44</td>
<td>48</td>
<td>40</td>
<td>40</td>
<td>52</td>
</tr>
</tbody>
</table>
Discussion

Analysis of the results of the BANA test for samples from bleeding and non-bleeding sites showed that the test was positive for 89 (43.63%) non-bleeding sites and for 30 (50.0%) bleeding sites. However, when the BANA-positive results for non-bleeding and bleeding sites were correlated and submitted to statistical analysis, no significant differences were observed (P > 0.05).

For the gingival index, there was a predominance of the 0.0, 1.0 and 2.0 scores among the children studied. It is interesting to note that, for the 264 sites studied, 204 samples were collected from non-bleeding sites and 60 from bleeding sites. The reason for this lack of proportion between the bleeding and non-bleeding sites is due to the fact that when the gingival index of the teeth examined presented scores of 0.0 and 1.0, there was no alternative but to collect from those sites.

With respect to this limited inflammation observed in children, Cox et al. (1974) attribute this phenomenon to reduced granulocyte migration compared with adults. In order to characterize the infiltrate in the connective tissue of inflamed gingiva in deciduous dentition, Longhurst et al. (1980) analyzed gingival tissue biopsies from 11 children aged 4 to 8 years. They observed the presence of large numbers of small and medium-sized lymphocytes, a distinct population of plasma cells and altered fibroblasts, as well as a marked loss of collagen, a small number of macrophages, mast cells and polymorphonuclear neutrophils, but rare T-cells (immunoblasts). On this basis, they concluded that, in the human species, the early stage of gingival disease occurs due to the presence of lymphocytes in the infiltrate, determined by T and B cells lines, and that gingival damage in children shares many aspects with early gingivitis damage in adults.

As to the predominance of GI scores of 0.0, 1.0 and 2.0, our data agree with those reported by Toledo and Sampaio (1970) and Moore et al. (1984) who only detected a prevalence of light or moderate gingivitis in children.

On the basis of negative/positive BANA data and non-bleeding and bleeding sites, an attempt was made to add the age factor. An explanation concerning the possible factors that influenced older ages, with BANA-positive tests, may be that these children were in a stage close to puberty, when the influence of hormonal factors may already be felt, as demonstrated by Orrico et al. (1991). When studying the interaction between BANA test, gingival status and age, these investigators noted a significant effect of age, with a greater risk of positivity to the test at bleeding sites at 11 (74.1%) and 12 (33.3%) years. We believe that further studies are needed to clarify this question and we suggest grouping younger ages, such as 5 and 6 years, and comparing their data with those obtained for age groups of 9 and 10 years. Studies of this kind may show a different behavior of the BANA-positive and BANA-negative correlation with bleeding and non-bleeding sites.

Conclusions

On the basis of the present results, we conclude that:
1. There was no statistically significant correlation between positivity to the BANA tests and bleeding and non-bleeding sites.
2. Age had no significant influence on the positivity of the BANA test as a function of bleeding and non-bleeding sites.

References

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