MICROBIOLOGICAL ANALYSIS OF PERIODONTAL PATHOGENS USING THE BANA TEST TO DIABETES MELLITUS PATIENTS

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ABSTRACT
The aim of study was to evaluate the periodontal condition of 15 non-diabetic patients (age range, 30-77 years) compared to 15 patients without diabetes. The BANA (N-benzoyl-DL-arginine-naphthylamide) test was used to analyze subgingival microbiota. The diabetic group was subdivided into three groups according to the degree of diabetic control: controlled, moderately controlled and poorly controlled. When the diabetic patients were subdivided and compared to the control group, significance was detected (P<0.01) in the total frequencies of the BANA scores (negative, moderately positive and positive: 1, 2, and 3, respectively) in the 5, 6-, and 7 mm pockets and also in the 4 mm pockets in the controlled group.

Keywords: periodontal disease progression, type II diabetes, probing depth, attachment loss, BANA test

INTRODUCTION
Periodontal disease involves an activity depending on factors such as type and pathogenicity of gingival sulcus bacterial plaque, host resistance and systemic involvement reflecting clinically as the presence of active areas. Bacterial plaque is the primary factor of periodontal disease and her composition is different both among individuals and among different sites of the same patient.(1,2)

The connection between oral health and systemic health is bidirectional; systemic illnesses, especially metabolic disorders, affect oral health, and it appears that oral health may affect systemic health.

Recent studies have revealed that a bidirectional relationship is evident between diabetes mellitus and periodontal disease.(3,4) Periodontal disease and diabetes are strongly interrelated and have common pathobiology. Inflammatory events during periodontal disease may play an important role in the development of diabetes and insulin resistance probably facilitates the progress of periodontal disease.

Loesche had analyzed the enzymes produced by periodontopathogenic bacteria, from the perspective for bacteriological monitoring. Three of the most important periodontopathogenic bacteria, Bacteroides gingivalis (Porphyromonas gingivalis), Treponema denticola, Bacteroides forsythus, and a species of the genus Capnocytophaga hydrolyze the synthetic substrate N-benzoyl-DL-arginine-beta-naphthylamide (BANA). (5,6,7)

Therefore there is a pressing necessity to establish the profile of oral microbial infection to form a scientific basis to inform and guide oral health, general medical, and public health practices to emphasize the role of routine health care for people with diabetes. To this end, the present study was
undertaken to establish the microbiological patterns of patients with chronic periodontitis in patients with diabetes compared with those without diabetes.

The study want to demonstrate that BANA test shows a significant correlation with the number of spirochetes present in subgingival plaque samples from several sites and from individual sites. Thus, BANA reaction can be used as an indicator of the spirochete level in individual sites of treated as well as non-treated patients.

**MATERIAL AND METHODS**

Material from 60 sites of 15 diabetic patients and from 60 sites of 15 control patients was collected.

We used the clinical methodology to determine the probing depths in diabetic patients as well as in control patients. The deepest pocket of each quadrant was selected for BANA analysis. 4 samples from each patient (one in each quadrant, 120 samples) were collected.

Bacterial plaque samples were collected using a Gracey 5/6 (Hu-Friedy) sterile periodontal curette to reach the deepest area of the periodontal pocket. BANA hydrolysis reaction was carried out with Perioscan Oral-B cards (Redwood City, CA, USA). The subgingival plaque sample was immediately placed on a reactive matrix on the lower portion of the card. Statistical analysis was carried out with the non-parametric chi square test.

**RESULTS**

Probing in 60 sites at patients with diabetes showed a prevalence of 37.8% for 5-mm pockets and 31.6% for 6-mm pockets. Probing depths of 4, 7, and 8 mm were observed less frequently (12.3%, 14.8%, and 3.7%, respectively) (Table 1).

At this patients the frequency of probing depths of 4 to 8 mm and the BANA test scores (values from 1 to 3) were correlated, 11 sites were negative (score 1, 18.33%), 15 sites were moderately positive (score 2, 25%), and 34 sites were positive (score 3, 56.67%) (Table 1).

### Table 1.

<table>
<thead>
<tr>
<th>Periodontal pocket depth (mm)</th>
<th>BANA test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Score 1</td>
</tr>
<tr>
<td><strong>Patients with diabetes</strong></td>
<td></td>
</tr>
<tr>
<td>4 mm</td>
<td>3</td>
</tr>
<tr>
<td>5 mm</td>
<td>4</td>
</tr>
<tr>
<td>6 mm</td>
<td>3</td>
</tr>
<tr>
<td>7 mm</td>
<td>1</td>
</tr>
<tr>
<td>8 mm</td>
<td>0</td>
</tr>
<tr>
<td>Number of sites</td>
<td>11</td>
</tr>
<tr>
<td><strong>Patients without diabetes</strong></td>
<td></td>
</tr>
<tr>
<td>4 mm</td>
<td>5</td>
</tr>
<tr>
<td>5 mm</td>
<td>4</td>
</tr>
<tr>
<td>6 mm</td>
<td>4</td>
</tr>
<tr>
<td>7 mm</td>
<td>2</td>
</tr>
<tr>
<td>8 mm</td>
<td>0</td>
</tr>
<tr>
<td>Number of sites</td>
<td>15</td>
</tr>
</tbody>
</table>
The percent of negative reactions (BANA score 1) and moderately positive reactions (BANA score 2) tended to decrease when the probing depth increased, that is, they were inversely proportional to the increase in probing depth. However, when BANA was positive (score 3) the relationship was directly proportional.

To control patients periodontal probing depths (4-8 mm) and BANA scores (values 1 to 3) of the 60 control sites showed that 15 sites (25%) were negative (score 1), 18 sites (30%) were moderately positive (score 2) and 27 sites (45%) were positive (score 3) (Table 1).

The percent of negative reactions (score 1) and moderately positive reactions (score 2) tended to decrease as probing depths increased. When BANA was positive (score 3) the relationship was directly proportional, that is, the percent of sites tended to increase as probing depth increased.

![Figure 1. BANA test values for patients with diabetes](image1)

![Figure 2. BANA test values for patients without diabetes](image2)
Using the non-parametric chi square test to verify if there was a correlation between the diabetic and the non-diabetic groups (control) in terms of different probing depths and BANA scores (negative, moderately positive and positive scores, 1, 2, and 3, respectively), no statistical significance was found (P>0.05).

DISCUSSIONS
Patients with diabetes increases the risk of periodontitis. (8) The link between diabetes and periodontal disease is increasingly recognized and that diabetics are more susceptible to periodontal disease. BANA hydrolysis by periodontopathogenic bacteria such as Porphyromonas gingivalis, B. forsythus and T. denticola and the genus Capnocytophaga is an alternative method for diagnosis of periodontal diseases. (9,10,11)

Our results for diabetic patients showed that the 4-8mm pockets were 56.67% with BANA positive scor 3. Statistically significant differences were observed (P>0.05) with the chi square test carried out with the total frequencies of the diabetic and control groups and BANA scores for 4, 5, 6, and 7 mm pockets. Our results confirm the etiological importance of Porphyromonas gingivalis (formerly B. gingivalis), and emphasize the possible participation of B. forsythus and T. denticola as etiological agents of periodontal disease in periodontal patients with diabetes.

CONCLUSIONS
Periodontal pathogens are distinctly different in patients with diabetes from those without diabetes. The differences are evident in both groups. These different microbiological characteristics are relevant also in dental and general medical practices.

REFERENCES
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