COMPARATIVE ANALYSIS OF PERIODONTAL PATHOGENS IN SMOKERS AND NON-SMOKERS WITH CHRONIC PERIODONTITIS – A MICROBIOLOGICAL STUDY

S. Gopalakrishnan, S. Parthiban, Uma Sudhakar

ABSTRACT

Aims: To compare the periodontal pathogens in smokers and non-smokers using, a non-invasive chair side technique, BANA (N-benzoyl- DL- arginine 2- napthylamide). Materials and Method: A total of 30 men were randomly selected out of which 15 are smokers and the rest 15 are non-smokers. Thirty sites in smokers and thirty sites in nonsmokers were selected for the study. Subgingival plaque samples from the selected sites in both Smokers and Non-Smokers were subjected to microbial examination. Results: Out of the 30 sites examined in smokers, (48.3%) showed BANA positive reactions compared to (16.7%) in non-smokers. Papillary bleeding score was taken in 30 sites in smokers and non-smokers. Similarly in our study Smokers with papillary bleeding score 3 had more BANA positive species (53.3%) than non-Smokers (26.7%). Conclusion: In conclusion the study result shows that there were more BANA positive species in smokers than non-smokers which is more significant (P -0.02%) and papillary bleeding score increases with BANA positive species.

Key Words: Chronic periodontitis; Smokers; Papillary bleeding score; BANA

Introduction

Periodontal diseases are a group of inflammatory conditions of the supporting tissues of the teeth that are caused by bacteria leading to a host-inflammatory response. This host response alone or together with virulence factors released from specific plaque bacteria result in soft tissue destruction and alveolar bone loss, the hallmark of periodontal diseases. Periodontal diseases represent a complex interaction between a microbial challenge and the host’s response to that challenge, both of which may be influenced by systemic and environmental factors such as diabetes and smoking. Epidemiologic evidence suggests that smoking may be the most important environmental risk factor impacting the development and progression of periodontal disease. Investigations regarding the association between smoking and periodontal disease have consistently demonstrated periodontal effects among smokers in comparison with nonsmokers.

The deleterious effects of smoking appear to result in part from a down regulation of the immune response to bacterial challenge. Smoking has many significant negative effects on PMN function, including impaired phagocytosis superoxide and hydrogen peroxide generation, integrin expression and protease inhibitor production. Alterations in gingival crevicular fluid and peripheral blood mononuclear cell levels of various cytokines in smokers, tipping the balance in favour of tissue breakdown, have been noted. Smoking decreases salivary IgA and IgG. There is specific reduction in IgG2 to a actinomyces comitans. This diminished production of protective antibodies may be due to the ability of tobacco products to decrease the proliferative capacity of T and B lymphocytes.

Although there is increasing evidence of specific bacterial etiology, identification of these organisms in different phases of disease process is technically challenging. With the increased understanding of the specific microorganisms implicated in different types and stages of human periodontal disease, microbiological observations of periodontal lesions have become more and more important. Identification of specific bacteria as an aid in determination of disease activity and effectiveness of clinical therapy has been frequently emphasized.

Gary. C. Armitage has reported various host and microbial mediators as potential markers for disease activity. He suggested that the detection of bacterial enzymes or enzymatic reactions directly in plaque associated with periodontal diseases may therefore be an easy and efficient method of diagnosis. Hence, the detection of selected bacterial species can be made by the presence of an enzyme that is unique to one or more of the clinically relevant species. A common approach is to expose a plaque sample to a substrate that can only be hydrolyzed by a specific enzyme. The hydrolysis of the synthetic peptide N-benzoyl- DL- arginine 2- napthyl amide (BANA) is dependent on the presence and number of bacteria in the plaque sample that possess enzymes capable of degrading it by way of their trypsin – like enzyme activity. The plaque species known to be BANA positive are p. gingivalis, Treponema denticola, Bacteroides forsythus (Presently named as Tannerella forsythia) and rarely capnocytophaga species. BANA test is a simple chair side diagnostic assay which enables the clinician to monitor the potential sites of active periodontal disease and even predict future attachment loss. It is useful for the dental profession as a diagnostic test for monitoring the levels of spirochetes in subgingival plaque samples. This study was conducted to compare the periodontal pathogens in smokers and non-smokers using, a non-invasive chair side technique, BANA (N-benzoyl- DL- arginine 2- napthylamide).

Materials and Method

A total of 30 men were randomly selected out of which 15 are smokers and the rest 15 are non-smokers. Thirty sites in smokers and thirty sites in nonsmokers were selected for the study. The following clinical parameters like Plaque index, Papillary bleeding score and Probing pocket depth were evaluated. After recording the above clinical parameters subgingival
plaque samples were collected with a gracey curette from both Smokers and non-Smokers. These samples were subjected to microbial examination using a chair side Diagnostic test (BANA).

Exclusion Criteria: Patients with aggressive periodontitis, Antibiotics for six weeks prior to examination, Patients who had received any periodontal treatment within six months prior to the present study, History of systemic disease that could influence the course of periodontal disease and Women.

Collection of plaque sample and sampling: Subgingival plaque samples were collected using separate sterile gracey periodontal curettes from two different sites from each patient. Each sample was then applied on the reagent matrix on the lower portion of the test strip in the location corresponding to the number of the tooth where the plaque sample was taken (Figure 2). The reagent strip was then placed in the incubator and then heated for 15 minutes at 55°C. The upper strip will show a dark blue colour, if it is positive and a faint blue colour, if it is weakly positive(Figure 3). The test results from the upper matrix were read as follows using the interpretation chart provided by the manufacturer.

### Results

A total of 60 sites were examined which included 30 sites in smokers and 30 sites in non-smokers with chronic periodontitis. The selected sites were assessed for the presence of BANA negative species i.e., *Treponema denticola*, *Porphyromonas gingivalis* and *Bacteroides forsythus*.

Out of the 30 sites examined in smokers, four sites (13.3%) showed BANA negative reactions, 11.5 sites (38.3%) showed weakly positive reactions and 14.5 sites (48.3%) showed positive reactions(Table 1). Similarly out of the 30 sites examined in non-smokers, 15 sites (50%) showed BANA negative reactions, 10 sites (33.3%) showed weakly positive reactions and five sites (16.7%) showed positive reactions. The result shows that there are more BANA positive species in Smokers compared to Non-Smokers (Table 1).

When papillary bleeding score was taken in 30 sites in smokers, 14 sites (46.7%) were scored as papillary bleeding score 2, 16 sites (53.3%) were scored as papillary bleeding score 3. Similarly, when papillary bleeding score was taken in 30 sites in non-smokers, 15 sites (50.0%) were scored as papillary bleeding score 2, eight sites (26.7%) were scored as papillary bleeding score 3, and seven sites (23.3%) were scored as papillary bleeding score 4. The result shows that the Papillary bleeding score increases with BANA positive species. (Table 2).

### Discussion

Periodontal disease is one of the most common diseases which have taken a significant amount of space in early historical writings. There is increasing evidence for specific bacterial etiology associated with various forms of periodontal disease. Hence, characterizing these species could have diagnostic values.

The organisms associated with chronic periodontitis are red complex and perhaps other microorganisms. In our study, we have used a chair side kit (BANA) a reliable method for diagnosing these red complex periodontal pathogens. Smoking has been shown to increase the severity and extent of periodontal disease. When combined with plaque-induced chronic periodontitis, an increase in the rate of periodontal destruction may be observed in patients who smoke and have chronic periodontitis. The early studies that examined the relation between smoking and oral cleanliness consistently found that Smokers have poorer oral hygiene than non-Smokers. However, some other studies reported significantly less plaque in smokers than non-Smokers. Smoking has important effect on oral bacteria, as Cigarette smoking could cause a lowering of the oxidation-reduction potential (Eh), and this could cause an increase in anaerobic plaque bacteria. Smokers harboured significantly higher levels and were at significant-

### Table 1 Comparison of BANA test

<table>
<thead>
<tr>
<th></th>
<th>BANA Negative</th>
<th>BANA Weakly Positive</th>
<th>BANA Positive</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N%</td>
<td>N%</td>
<td>N%</td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td>15 50%</td>
<td>1033.3%</td>
<td>0516.7%</td>
<td>0.02</td>
</tr>
<tr>
<td>Smokers</td>
<td>4 13.3%</td>
<td>11.538.3%</td>
<td>14.548.3%</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Papillary Bleeding Score

<table>
<thead>
<tr>
<th>Papillary Bleeding Score</th>
<th>Group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-Smokers</td>
<td>Smokers</td>
</tr>
<tr>
<td></td>
<td>N%</td>
<td>N%</td>
</tr>
<tr>
<td>2</td>
<td>1550.0%</td>
<td>1446.7%</td>
</tr>
<tr>
<td>3</td>
<td>0826.7%</td>
<td>1653.3%</td>
</tr>
<tr>
<td>4</td>
<td>0723.3%</td>
<td>-----</td>
</tr>
<tr>
<td>Total</td>
<td>30100.0%</td>
<td>30 100.0%</td>
</tr>
</tbody>
</table>
ly greater risk of infection with Bacteroides for sythesis than non-smokers.\textsuperscript{18} Adjusting for disease severity, Porphyromonas Gingivalis was also more likely to subgingivally infect smokers than non-smokers.\textsuperscript{19}

The results in our study clearly demonstrated significant correlation exists between the BANA test and Smokers (P - 0.02). As the probing pocket depth increased in smokers, there were more positive reactions to BANA test clearly suggesting the etiology of the BANA positive species associated with increasing probing depth and attachment loss. These results are similar with the data reported by others.\textsuperscript{20,22} The reduced bleeding in smokers could conceivably lead to a lower oxygen tension in the pocket environment. Smoking also has profound effects on the immune system. Locally the vasoconstrictive effect of nicotine causes a reduction in the gingival blood flow, possibly decreasing the number of cells, the amount of oxygen and blood constituents that reach the gingiva. Smoking can depress the primary and secondary immune responses and is associated with reduced levels of circulating antibody. The chemotactic and phagocytic activities of oral PMNs are depressed by smoking directly weakening host defense response.\textsuperscript{23} Smoking may also interfere with the inflammatory process by affecting the release of pro-inflammatory cytokines. These factors could be the reason for the increased BANA positive species in the Smokers (48.3%) compared to non-Smokers (16.7%) in our study.

In the present study, the papillary bleeding score was recorded according to Loesch’s criteria(1979).\textsuperscript{24} The results demonstrated strong correlation between papillary bleeding score (PBS) and BANA test in Smokers (46.7% in PBS 2) and (53.3% in PBS 3) and it can be inferred that there were more BANA positive results as the papillary bleeding score increased. In present study the papillary bleeding score of 4 was absent. The reason for the low bleeding tendency in smokers has been attributed to nicotine effect, in that chronic low doses of nicotine can cause a sustained peripheral vasoconstriction and decreased peripheral blood supply to the tissues of the periodontium, which might manifest clinically as inflammation without bleeding. Many authors investigated the relationship between the prevalence of periodontal pathogens in subgingival plaque samples of periodontal pockets and periodontal status using polymerase chain reaction techniques.\textsuperscript{25-27} It was found that sites in which all three microorganisms were detected were bleeding on probing positive and had greater pocket depths than those where only one or two species were found.\textsuperscript{22} Similarly in our study smokers with papillary bleeding score 3 had more BANA positive species (53.3%) than non-smokers (26.7%) which shows that there were more amount of these red complex organisms (Porphyromonas gingivalis, Treponema denticola, and Bacteroides forsythus) in the bleeding sites of periodontal pockets in Smokers than non-Smokers.

**Conclusion**
The major factor leading to greater plaque accumulation in smokers is inadequate oral hygiene. Tobacco smoke has a strong reducing capacity in the mouth and appears to contribute to anaerobiosis. This predisposes Smokers to oral infection by anaerobic organisms. The presence of Porphyromonas gingivalis, Bacteroides forsythus, and treponema denticola was significantly associated in smokers with chronic periodontitis compared to non-smokers in our study. These findings highlight the importance of microbial studies in different population groups which may give different results in terms of the compositions of subgingival microbiota and their relationship with periodontal status. Further studies with larger sample size, using PCR could provide us on the accuracy of these periodontal pathogens in Smokers.

**Authors Affiliations**
1. S. Gopalakrishnan MDS, Senior Lecturer, 2. S. Parthiban MDS, Senior Lecturer, 3. Uma Sudhakar MDS, Professor, Department of Periodontics, Thai Moogambigai Dental College, Chennai, Tamilnadu, India.

**References**
Comparative analysis of periodontal pathogens in smokers and non-smokers


21. de Moraes GRISI MF, Novaes AB, Ito IY, de Souza SALVADOR SL. Relationship between clinical probing depth and reactivity to the BANA test of samples of subgingival microbiota from patients with periodontitis. Braz Dent J. 1998;9(2):77-84.


How to cite this article

Address for Correspondence
Dr. S. Gopalakrishnan MDS, Senior Lecturer, Department of Periodontics, Thai Moogambigai Dental College, Chennai, Tamilnadu, India.
Email: gopalakrishnan_perio@ymail.com

Source of Support: Nil
Conflict of Interest: None Declared