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Relationship between Periodontal Inflamed Surface Area [PISA] and anaerobic periodontal infections assessed by BANA [N-benzoyl-DL- arginine-β-napthylamide] assay

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Abstract: Background: chronic periodontitis is an inflammatory disease of the periodontium caused by microbial infection. The BANA test is a simple chairside test which allows the clinician to quantify the amount of anaerobic periodontal infection. PISA quantifies the inflammatory burden posed by periodontitis to cause systemic inflammatory response. By comparing the BANA test values with PISA, the validity of PISA as a tool to quantify the amount of inflamed periodontal tissues can be analysed.

Aim: to compare PISA values with anaerobic periodontal infection assessed by BANA assay.

Patients and Methods: A total of 80 sites were selected [40 each from 10 patients in healthy group and 10 patients in chronic periodontitis group]; after measuring the probing depth/clinical attachment levels, the tooth with deepest probing pocket depth from each sextant was selected, and the plaque sample was collected for BANA assay and the corresponding PISA values were calculated.

Results: The Mann-Whitney test was used to compare the BANA test results with PISA values in healthy and periodontitis groups. There was a significant difference [$p \leq 0.0001$], in PISA values from BANA positive sites compared to BANA negative sites in both the groups.

Conclusion: PISA values can be considered as indicators of anaerobic periodontal infection, which clearly demonstrates the validity of PISA in quantifying the inflammatory burden.

Keywords: periodontal inflamed surface area, anaerobic periodontal infection, N-benzoyl-DL- arginine-β-napthylamide[BANA] test.

I. INTRODUCTION

Periodontal disease comprises of a group of inflammatory conditions of the supporting tissues of the teeth, which is characterized by loss of connective tissue attachment and alveolar bone resulting in the formation of probeable pockets around the teeth.

Periodontal microbiota are a complex community of micro-organisms which play a primary role in the initiation and progression of the periodontal disease.Although several distinct bacterial species are found in the oral cavity, only a finite number of species appear

to be associated with the clinical disease[1]. A limited number of bacterial species such as P.gingivalis[2],T.forsythia[3],T.denticola[4] and other spirochetes are associated with most forms of chronic periodontitis. Also this microbial complex is strikingly related to clinical measurements of periodontal disease.

Most of the suspected periodontal pathogens are gram negative anaerobes and use proteins and peptides as nutrients. Such anaerobic infections could be diagnosed by detecting enzyme activity directed towards proteins and peptides. One such enzyme, capable of hydrolyzing the synthetic trypsin substrate N-benzoyl-DL-arginine-β- napthylamide [BANA] is a trypsin- like enzyme [BANA hydrolyzing enzyme].

P.gingivalis,B.forsythus and T.denticola species which are putative periodontal pathogens which possess BANA hydrolytic activity[5]. This BANA test has been designed into a commercially available,solid-state assay[BANAMET LLC,Ann Arbor, MI USA] which is a simple, chairside diagnostic test. It provides useful information regarding the intensity of anaerobic infection associated with periodontal disease.

The great variation in periodontal classification used in the various studies and the lack of a tool that adequately assesses the inflammatory burden of periodontitis is a major drawback of the studies published on the periodontal inflammation systemic disease interaction. Therefore, a new measure of periodontitis as a risk factor for other diseases was developed, the PISA. PISA quantifies the amount of inflamed periodontal tissue, thereby quantifying the inflammatory burden posed by periodontitis[6].

Thus by comparing the PISA values with BANA test results the validity of PISA as a tool to assess the inflammatory burden can be determined. In this perspective this study was carried out to analyse the validity of PISA.

II. MATERIAL AND METHODS

The subjects for this study were selected from the patients attending a private clinic in Chennai.

The patients were selected on the basis of clinical examination using the following criteria[7].

1. Eighty sites were selected [fourty sites each from,ten patients in healthy control group consisting of patients with no/minimal signs of inflammation and ten patients from the periodontitis group consisting of patients suffering from chronic generalized form of periodontitis with probing pocket depth/attachment loss ≥ 5mm were selected.
2. Above the age group of 35 years.
3. Free from systemic diseases.
4. Who have not received any local or systemic antimicrobial and anti-inflammatory therapy for the past six months.
5. Who had not received any periodontal treatment for atleast one year before examination.

6. Pregnant women and women who were on contraceptive medications were not selected for the study.

III. PROCEDURE

After measuring the probing depth/clinical attachment levels, the tooth with deepest probing pocket depth from each sextant was selected[a total of 80 sites were selected 40 sites from the healthy group and 40

sites from the chronic periodontitis group], and the plaque sample was collected for BANA assay and correspondingly the PISA values were calculated using bleeding on probing, attachment levels and probing depth measurements.

Sub-gingival plaque collection and sampling:

Name and age of the patient along with information of described site were recorded in the reagent strip.

Supragingival plaque was removed and discarded prior to sampling. Subgingival plaque material was collected using periodontal curettes positioned in the deepest areas of the periodontal pockets.

Each sample was applied on the raised reagent matrix affixed to the lower portion of the test strips in the location corresponding to the number of the tooth from where the specimen was taken.

After sampling the desired sites, the upper test matrix was moistened with distilled water using a sterile cotton swab.

The reagent strip was folded at the perforation so that lower and upper matrices come in direct contact with each other. The reagent strip was then placed in the incubator for 15 minutes at 55^0c.The lower portion of the test strip was separated from the upper strip and discarded. After incubation, the test results [colour reaction] from the matrix were read as follows, using the following BANA reagent interpretation chart.following BANA reagent chart.

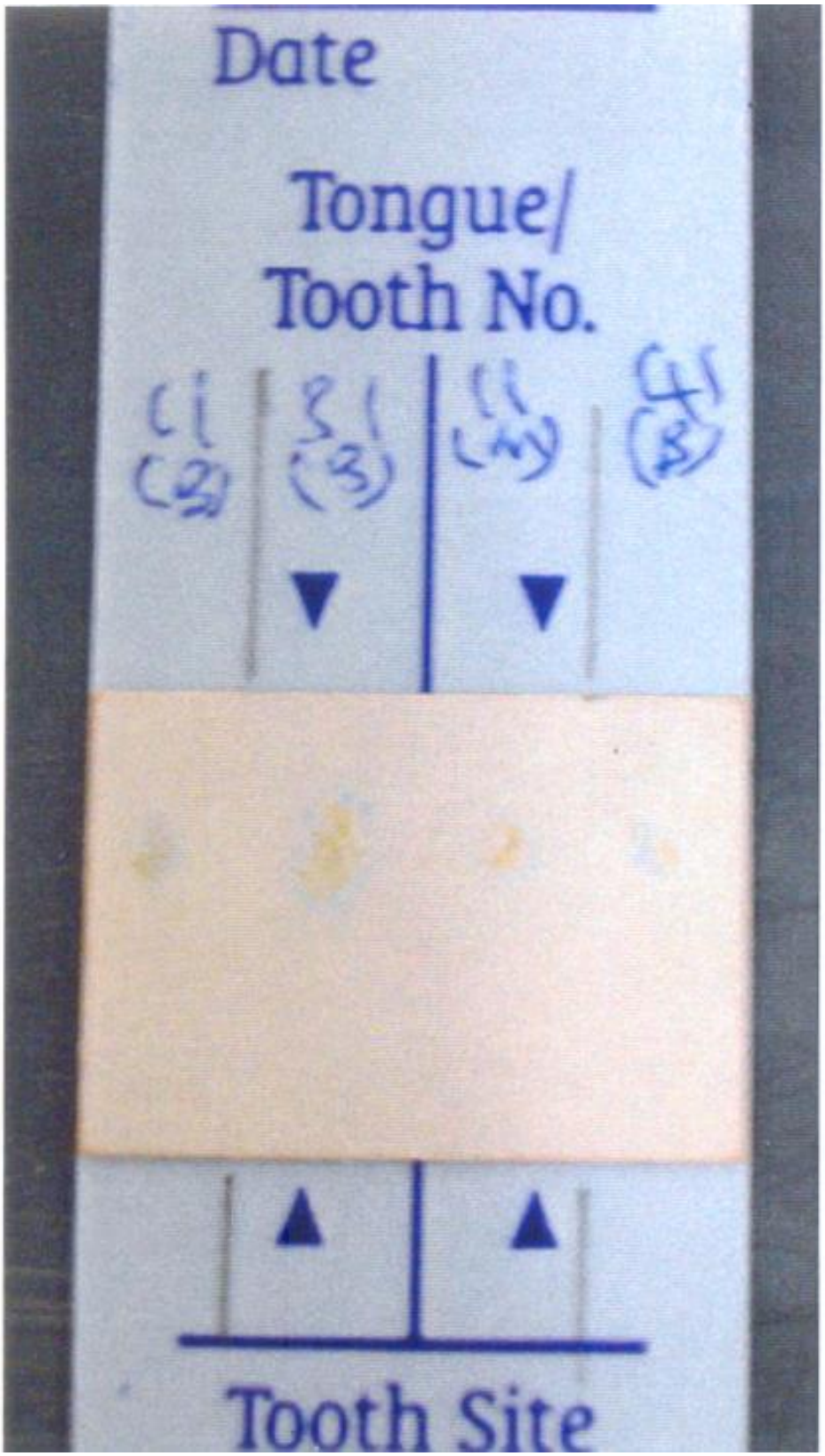


Fig (1) BANA reagent strip after incubation [colour change seen in upper matrix]

Negative: no blue color is observable on a pale red background.

Weak positive: faint blue color on a pale red background.

Positive: distinct patches of blue somewhat larger and darker than weak positive reaction on a pale red background.

PISA was calculated for the corresponding tooth by filling in clinical attachment level, recession and bleeding on probing on six sites per tooth in the freely downloadable spread sheet available from www.parsprototo.info.

The average of PISA scores of the healthy and periodontitis group was calculated and the results were compared with BANA test results of the corresponding groups.

IV. Statistical Analysis

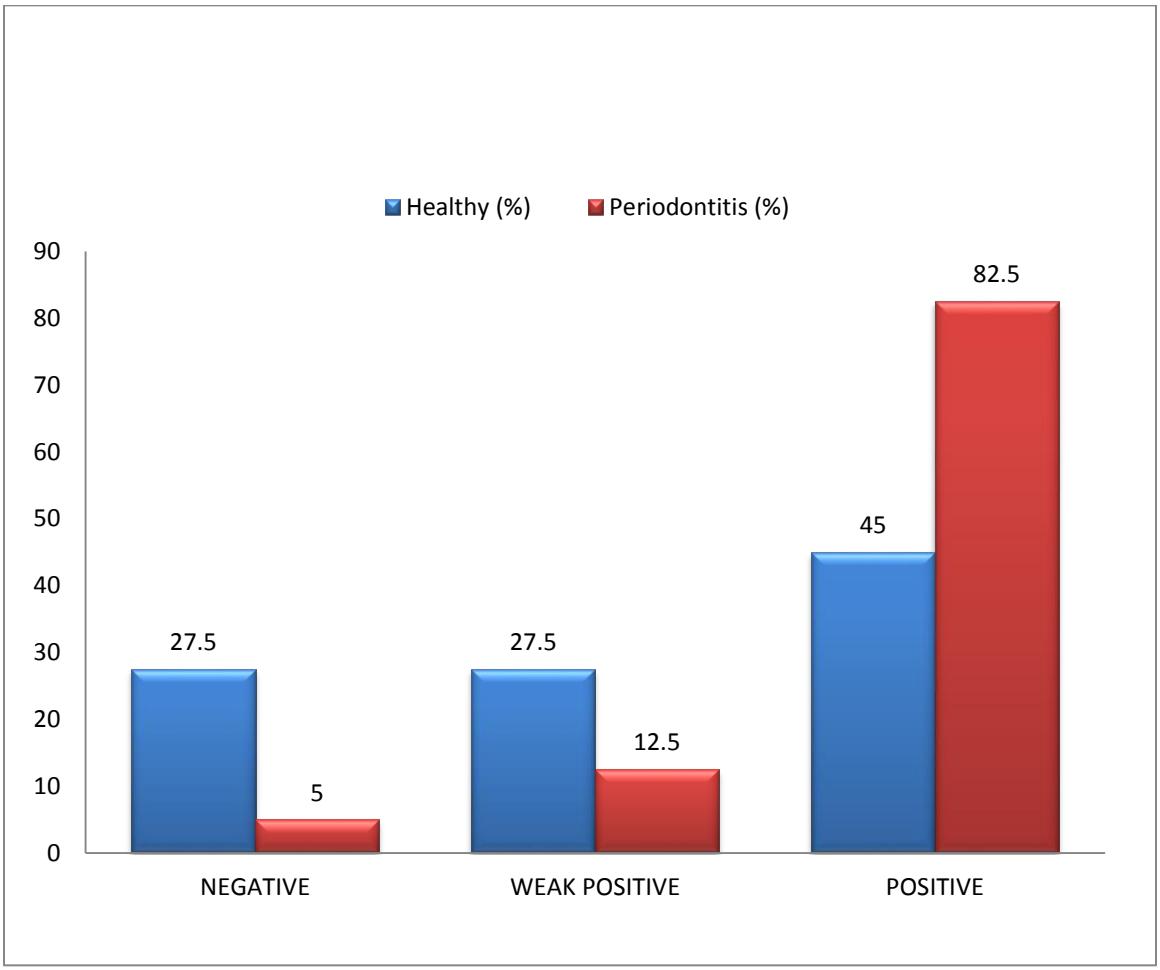
The collected data was analysed with SPSS 17.0 version. To describe about the data the descriptive statistics, mean and standard deviation was used. Frequency analysis was used to find the distribution of BANA scores for a independent bi-variate analysis, the non-parametric mann-whitney test was used with significance level of $p \leq 0.05$.

Results:

Table 1

BANA Score Comparison

Groups	NEGATIVE	WEAK POSITIVE	POSITIVE
Healthy [%]	27.5	27.5	45
Periodontitis [%]	5	12.5	82.5



Fig(2) Bana Score Comparison

Table 2

Comparison of BANA with PISA

BANASCORE COMPARISON WITH PISA USING MANN-WHITNEY TEST							
	Groups	NEGATIVE	WEAK POSITIVE	POSITIVE	MANN-WHITNEY	P-VALUE	SIG
BANA SCORE	Healthy	11[27.5%]	11[27.5%]	18[45%]	3.568	0.000	S**
	Periodontitis	2[5%]	5[12.5%]	33[82.5%]			
PISA	Healthy	14.31 ± 10.11	16.07 ± 7.60	23 .32 ± 20.65	7.458	0.000	S**
	Periodontitis	77.1 ± 41.57	76.7 ± 22.49	99.2 ± 44.45			
S** denotes significant at P < .0001							

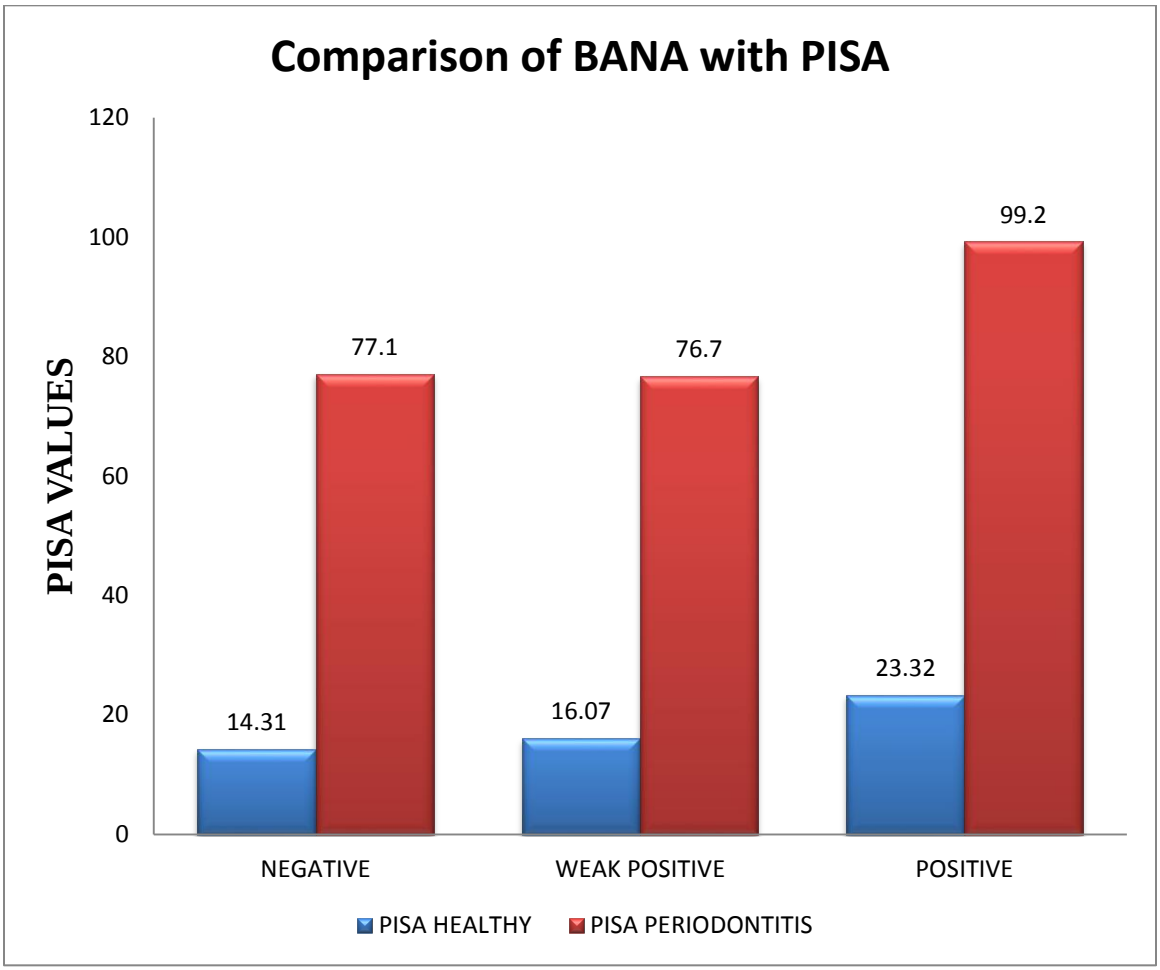


Fig (3) Comparison of BANA scores with PISA

V. Discussion

The objective of the present study was to check the validity of the PISA classification system, by comparing the levels of putative periodontal pathogens[BANA test results] which are the primary etiological factors for chronic periodontitis with PISA values.

Chronic periodontitis is an inflammatory disease of the periodontium caused by microbial infection. Although several distinct bacterial species are present in the oral cavity, a limited number of bacterial species such as Porphyromonas gingivalis, Tanerella forsythia, Treponema denticola and other spirochetes are associated with most forms of chronic periodontitis, also they are strikingly related to clinical measurements of periodontal disease.

BANA test[BANAMET LLC] format was used because it is helpful as a simple objective chair side test to find out the presence of putative periodontal pathogens[Porphyromonas gingivalis, Tanerella forsythia, Treponema denticola]. The limitation of BANA test is, it does not identify which of the three BANA positive species is present in the plaque. However, as all the three species are anaerobes, it allows the clinician to determine that an anaerobic infection is present[8], hence it was used in the present study.

The need to quantify the amount of inflamed periodontal tissue in order to quantify the inflammatory burden to establish the role of inflamed periodontal tissue in eliciting bacteraemia, systemic inflammatory responses or cross-reactivity[9] led to the development of PISA, a classification of periodontitis that quantifies the amount of inflamed periodontal tissue and, as such quantifies the systemic inflammatory burden. The limitations of PISA are , first the parameters [CAL, recession and BOP measurements] always include measurement errors related to observer, instrument, teeth, patients and their interactions, second the formulas transforming CAL recession to surface area, use population- based mean values of both root surface areas and root lengths. Thus, individual variations in root surface area and root length are not taken into account when calculating PISA and third PISA quantifies the amount of inflamed periodontal tissue in two dimensions, whereas in fact periodontitis is a three dimensional inflammatory process, i.e. extending into the connective tissue around the root.

For these reasons, PISA may not precisely quantify the the amount of inflammatory tissue. However, PISA likely quantifies the amount of inflamed periodontal tissue for each individual patient more accurately than any classification currently used, hence it was used in the present study.

Out of the 80 sites examined 13 sites showed negative BANA results [11 sites from the healthy group and 2 sites from the chronic periodontitis group]. The negative BANA test reactions in healthy sites may be due to the presence of putative microorganisms below the detection level of BANA test and/or presence of non-putative microorganisms. A negative BANA result indicates that the BANA positive organisms are below the range of 10,000 to 1,00,000 colony forming units[cfu] at the site of sampling[10].

16 sites showed weak positive reactions[11 sites from healthy group and 5 sites from chronic periodontitis group]. A weak positive reaction indicates that the BANA positive species are above the level of 1,00,000 cfu. The presence of a weak positive reaction may be explained by the ability of the BANA test to identify the microbial colonization of pre-clinical infection.[11]

51 sites showed BANA positive reactions [18 from healthy group and 33 from chronic periodontitis group]. A positive BANA reaction indicates that the BANA positive species are present in high proportions in the sample. The positive BANA test reactions in healthy group may be explained by the fact that, even though pocket depth was small, clear gingival changes were present and the site may have been colonized by BANA positive microorganisms[12].

When the PISA values were compared with BANA test results, there was a significant difference in PISA values between BANA positive and BANA negative sites.[23.32+20.65 and 14.31+10.11 for healthy group and 99.2+44.5 and 77.1+41.57 for chronic periodontitis group]. The results clearly demonstrate increased PISA values from BANA positive sites from both healthy and chronic periodontitis group compared to PISA values from BANA negative sites from both healthy and chronic periodontitis group, which clearly demonstrates the association between increased load of anaerobic microbial infection and periodontal inflammation.Thus PISA

values can be considered as indicators of anaerobic periodontal infection, which adds weight to the validity of the PISA classification system and hence PISA apart from being used to quantify periodontal inflamed surface area can also be used as a valid clinical tool to quantify anaerobic periodontal infection.

Further studies correlating certain oral microorganisms that might play a key role in causing systemic diseases[e.g. campylobacter rectus, prevotella intermedia, porphyromonas gingivalis and peptostreptococcus micros][13] with PISA might help in predicting the probability of periodontitis to cause other diseases, thereby helping to establish a biological plausibility for the role of periodontitis in causing systemic disease.

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