Original Research Article

Using of BANA-Enzymatic™ test kit to detect periodontal heath of patients with generalised chronic periodontitis before and after scaling and root planing – A randomized control study

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Abstract

Background: Clinical improvements after SRP are associated with microbiological changes that include a decrease in microbial load and a mean percentage change of certain periodontal pathogens, such as *Treponema denticola*, *Porphyromonas gingivalis and Tarnella forsythus.* These species are gram negative anaerobes which possess, in vivo an enzyme capable of hydrolyzing synthetic trypsin substrate, BANA (N-Benzoyl D-L Arginine -2 Naphalamide). BANA a colorless substrate, it releases β- naphthylamide, which turns orange red when a drop of fast garnet is added to the solution. Several Bacteroides and Capnocytophaga species were occasionally BANA positive, only when in large CFU's. Loesche proposed the use of this BANA reaction in subgingival plaque samples to detect the presence of any of these periodontal pathogens and thus serve as a marker of disease activity.

Aim and objective: The aim and objective of this study was to detect the presence of BANA micro-organisms and also to determine the effect of scaling and root planning in adult periodontitis patients.

Materials and Methods: 30 Subjects randomly selected comprising of both the sexes, visiting outpatient department of Periodontology, Govt. Dental College and Hospital Srinagar, were considered for the present clinical study after meeting inclusion and exclusion criteria. Subjects were randomly assigned into two groups:- 15 Subjects in Control Group (Group A) and 15 Subjects in

Treatment Group (Group B) i.e. The Control group- Group A Treatment group- Group B Four test sites were selected from each patient and assessed for plaque index, bleeding index and pocket depth and attachment loss before and after scaling and root planning. BANA test was used for the detection and prevalence of the "red complex" bacteria in plaque samples.

Results: The BANA tests are statistically correlated with the severity of periodontal destruction. There was a statistically significant correlation between the BANA test results and the parameters used to test the periodontal heath.

Conclusion: This study encourages the use of BANA as chair-side tests for a proper diagnosis of periodontal condition. It also gives a picture of microbiological flora of the plaque.

Key words

N-benzoyl-DL-arginine-2-napthylamide test, Periodontitis, Red complex.

Introduction

Periodontal diseases are chronic inflammatory conditions characterized by loss of connective tissue, alveolar bone resorption and formation of periodontal pockets as a result of the complex interaction between pathogenic bacteria and host immune response [1-3]. Periodontitis is result of cumulative exposure of dental plaque, thus the main aim of periodontal therapy is the prevention of plaque accumulation and maintain periodontal health. The clinical effect of scaling and root planing (SRP) are well documented [4-6]. These studies indicated that SRP decreased pocket probing depth and attachment level measurements particularly at the deeper sites. Microbiological studies on effect of SRP indicated that proportion of spirochetes and motile rods decline after SRP while cocci and non-motile rods increased [7]. Haffajee, et al. reported that SRP alone has limited effect on some pathogenic species [8].

Clinical improvements after SRP are associated with microbiological changes that include a decrease in microbial load and a mean percentage change of certain periodontal pathogens, such as *Treponema denticola*, *Porphyromonas gingivalis* and *Tarnella forsythus* [11].These species are gram negative anaerobes which possess, in vivo an enzyme capable of hydrolyzing synthetic trypsin substrate, BANA (N-Benzoyl D-L Arginine -2 Naphalamide) .BANA a colorless substrate, it releases β- naphthylamide, which turns orange red when a drop of fast garnet is added to the solution. Several Bacteroides and Capnocytophaga species were occasionally BANA positive, only when in large CFU's [12]. Loesche proposed the use of this BANA reaction in subgingival plaque samples to detect the presence of any of these periodontal pathogens and thus serve as a marker of disease activity [12].

BANA-Enzymatic test™ kit (Ora Tec Corporation, Manassas, USA) is a rapid and reliable chair side diagnostic test, which can be performed in about 15 min time, that can give information about the presence of three of the putative pathogens in subgingival plaque samples, that is, *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* that share unique ability of hydrolyzing the trypsin substrate, BANA.

Materials and methods

30 Subjects randomly selected comprising of both the sexes, visiting outpatient Department of Periodontology, Govt. Dental College and Hospital Srinagar, were considered for the present clinical study after meeting inclusion and exclusion criteria.

Inclusion criteria
The criteria for inclusion in the study were:
- Patients of age between 25-50 years.
- Patients diagnosed as suffering from generalized chronic periodontitis

determined on clinical and radiographic examination

- Minimum of 4 teeth with one site with pocket depth ≥5 mm and ≤7 mm
- Cooperative patients who are able to attend the hospital at frequent intervals.

Exclusion criteria

- Patient who had received any type of invasive periodontal therapy for past 4 months.
- Presence of any systemic disease that would influence the course of periodontal disease.
- Pregnancy and lactation.
- Smoking habit.
- Allergic to chlorhexidine
- Subjects having periapical lesions, gingival abscess , periodontal abscess
- Patients with history of antimicrobial drug intake for 7days or longer in previous 3 months

Before the selected subjects were taken up for the study, they were made to sign a written consent from regarding the benefits and protocol of the study.

Recording of periodontal parameters
Four-Non adjacent periodontal pockets in posterior segment of mouth measuring depth ≥5 mm and ≤7 mm were assessed. Following periodontal parameters will be recorded in both groups (Group I and Group II) at baseline:

1. Plaque index (Sillness and Loe, 1964) [9].
2. Sulcus bleeding index (Muhlemann H.R and son, 1971) [10].
3. Probing Pocket depth. (with Williams Graduated Periodontal Probe) [11].
4. Relative Attachment level i.e. distance between base of sulcus or pocket and a fixed reference point (horizontal notch) on the acrylic stent [11].
5. BANA Test (*N*-benzoyl-*d* L-arginine-2-napthylamide) [12]

Treatment procedure

The Control group (Group A) Received oral hygiene instructions. Treatment group (Group B - SRP + 0.2% CHX) full mouth scaling using ultra-sonic scaler (magnetostrictive) followed by root planing using Gracey curettes performed under local anesthesia if required. Received oral hygiene instructions and were on follow up till 42nd day.

Statistical methodology employed
Statistical analysis was done by using Statistical Software SPSS (Version 20.0) and Microsoft Excel. Data was analyzed by applying descriptive statistics viz., means, standard deviations and percentages and presented by means of Bar Diagrams. Inter group analysis was done by applying Student's Independent t-test and Chi-square test. For intra group analysis, Paired t-test and Cochran's Q-test were employed. P-value less than 0.05 was considered statistically significant.

Results

Of the 30 subjects selected, the mean baseline clinical parameters for the two subject groups were tabulated:

Plaque Index: Mean plaque index of control group (Group A) baseline was 1.8805 day 42 was 1.2195. Mean plaque index of test group (Group B- SRP +CHX) at baseline was and 42 day was 0.9871. On comparison between the two groups at the baseline the difference was statistically not significant. The test group (group B) showed greater improvements in plaque control index scores than control group (group A). The difference in results showed a statistically significant at day 42 (p < 0.001).

Sulcus Bleeding Index: Mean sulcus bleeding index of control group (Group A- SRP +PLACEBO) at baseline was 1.8805 at day 42 was 0.9479. Mean of test group (Group B- SRP +CHX) at baseline was 1.9168 at 42nd day was 0. On comparison between the two groups at the baseline the difference was statistically not significant. The test group (group B) showed

greater improvements in sulcus bleeding index scores than control group (group A). The difference in results showed a statistically significant decrease at day 42 ($p < 0.001$).

Probing Pocket Depth: Mean probing pocket depth in control group (Group A) at baseline was 6.82, at day 42 was 4.31. Mean probing pocket depth in test group (Group B) at baseline was 6.89, at 42 day was 3.90. On comparison between the two groups at the baseline the difference was statistically not significant. Whereas at day 42nd the test group (group B) showed greater improvements in mean probing pocket depth scores than control group (group A). The difference in results showed a statistically significant decrease of probing depth at day 42 (p 0.013).

Relative Attachment Levels: Mean relative attachment level in control group (Group A) at baseline was 9.82, at day 42 was 7.26. Mean relative attachment level in test group (Group B) at baseline was 9.89, at 42 day was 6.93. On comparison between the two groups at the baseline. The difference was statistically not significant. The test group (group B) showed greater reduction in mean relative attachment levels or the mean gain of attachment than

control group (group A) on 42nd day. The results showed a statistically significant difference at day 42 (p 0.012).

BANA Test: In group A, at baseline percentage of sites with score 0 (negative result) was 0, at 42nd day the percentage was increased to 65%. The percentage of sites with score 2 (positive result) was 85.5% at baseline and decreased to 13.3% on 42 day. The difference in the scores are statistically highly significant (<0.001). In group B, at baseline percentage of sites with score 0 (negative result) was 0, at 42 day the percentage was increased to 73.3%. The percentage of sites with score 2 (positive result) was 83% at baseline and decreased to 6.7% on 42 day. The difference in the scores are statistically highly significant (<0.001).

On comparison, the results dictated that the improvement in the results were statistically significant (p 0.017) on the 42nd day and non-significant on the baseline. The test group showed a significant increase in the BANA negative sites in comparison to the control group on the 42nd day. The no of BANA positive sites were also statistically decreased more in test group than in the control group.

Table - 1: Changes in BANA test score in Group A

BANA	Baseline		42 Days		P-value
	No.	%	No.	%	
0	0	0.0	39	65.0	<0.001*
1	9	15.0	13	21.7	
2	51	85.5	8	13.3	

*Statistically Significant Difference (P-value by Cochran's Q-test)

Table - 2: Changes in BANA test score in Group B.

BANA	Baseline		42 Days		P-value
	No.	%	No.	%	
0	0	0.0	44	73.3	<0.001*
1	10	16.7	12	20.0	
2	50	83.3	4	6.7	

*Statistically Significant Difference (P-value by Cochran's Q-test)

Table - 3: Comparison between two groups based on BANA test score.

	BANA	Group A (n=54 sites)		Group B (n=54 sites)		P-value
		No.	%	No.	%	
Baseline	0	0	0.0	0	0.0	0.749#
	1	9	15.0	10	16.7	
	2	51	85.5	50	83.3	
42 Days	0	39	65.0	44	73.3	0.017*
	1	13	21.7	12	20.0	
	2	8	13.3	4	6.7	

*Statistically Significant Difference (P-value by Chi-square test)
#Statistically Non-significant Difference (P-value by Chi-square test)

Table – 4: Comparison of validity test of BANA-Enzymatic™ test results.

	Sensitivity	Specificity	Overall accuracy
Bleeding index	83.41	26.47	68.52
Plaque index	92.17	36	70
Pocket depth	95.13	75.22	89.66
Relative attachment	95.17	74.23	89.83

The clinical baseline characteristics were similar between the two groups. The score of the sites exhibiting high plaque index score, sulcus bleeding score as well as mean PD and RAL were significantly reduced at 42nd day post-therapy in both treatment groups. Even though no differences in the clinical parameters were observed between the two groups at baseline, the percentage of sites with visible plaque, sulcus bleeding, mean PD and RAL were significantly lower in the test group at 42 days post-therapy. The baseline microbiological data analysis showed that the two groups were homogeneous as regards the distribution of the BANA results. After SRP, 42 days post-therapy, however, group B showed a greater frequency of sites with BANA-negative results compared with control group. Both groups had a reduced frequency of BANA-positive sites and increased frequency of BANA-negative sites (**Table – 1 to 4**).

Discussion

Periodontal infection is a Gram-negative, anaerobic oral infection. The bacteria responsible for this condition are capable of producing a variety of biochemical inflammatory markers that directly affect the host. Bacterial plaque contributes to the periodontal breakdown by direct injury to tissue and by stimulating host mediated responses that results in tissue destruction; direct injury is caused by both endotoxins and exotoxins produced by the bacterial mass and enzymes, secreted by bacteria to facilitate its penetration by breaking down the structure barrier [13].

Considerable interest has been developed in methods of detecting periodontopathogens in plaque samples with the development of the diagnostic systems [14]. A diagnostic test should be useful, ideally leading to a choice of treatment that would confer benefits upon the patient. It should be simple and aimed at identifying certain forms of periodontal disease associated with some predominant periodontopathogens, which possess few common characteristics. Instead of identifying individual bacterial species on the basis of these common characteristics, a group of micro-organisms can be detected for diagnosis of periodontal disease. Here, BANA-Enzymatic test™ screen subgingival plaque samples for trypsin-like proteolytic activity that is common to only few known periodontopathogens such as *P. gingivalis*, *T. denticola*, and *T. forsythia*.

Most of the clinical studies showed a strong relationship between a BANA positive reaction and high levels of plaque spirochetes [12]. However, there are possibilities that other plaque species and host enzyme could be contributing to this reaction. Screening of 255 strains from 51 species does not hydrolyze BANA that is, demonstrated a uniform BANA negative reaction, and several *Capnocytophaga* species were variable in giving a weak BANA reaction. Though BANA hydrolyze activity was found in *Capnocytophaga* species, it could be associated with the BANA reaction because *Capnocytophaga* species were found in very low proportions in both the BANA positive and BANA negative results.

It had been seen that BANA hydrolysis by plaque samples has the potential to be the marker of periodontal morbidity as assessed by probing depth measurements and by plaque proportions of spirochetes [15].

A positive BANA test indicates more spirochetal load than bacterial load, as indicated by the ability of the BANA test to identify subgingival plaque with elevated spirochetes, but not elevated bacteria in the treated patients. Bretz and Loesche agreed with the previous data, which showed that BANA positive test in subgingival plaque occurred only when appreciable levels of spirochetes were present [16].

Limitation of the BANA test is, firstly, it does not identify which of the three BANA positive species is present in plaque, but since they all are anaerobic species, it should enable the clinician to diagnose an anaerobic infection, and only such a diagnose could be useful for the treatment and management of periodontal disease of the patient. Secondly, to preserve the shelf life of the test strips, ensure that top to the dispensing bottle is tightly closed on the bottle after removing a test strip, and the desiccant is contained within the dispensing bottle. Thirdly, the unavailability of this kit in India.

The presence of a BANA positive plaque around a tooth site at the conclusion of the initial periodontal treatment indicates still presence of higher proportions of anaerobic bacteria in the periodontal pocket as a residual infection. This is also indicative of the future attachment loss, which is having potentially greater clinical significance [16].

N-benzoyl-DL-arginine-2-napthylamide test kit result is showing weak positive reaction in four sites before treatment exhibited a positive response after initial periodontal therapy. It had been shown that conversion of BANA positive plaque to BANA negative plaque, leading to the reduction in the need of surgical intervention was significantly modified by certain host factors. These host factors are host immune responses or patient's ability to maintain the oral hygiene at an optimum level. All these intrinsic and extrinsic host factors determine the tooth site-specific response to a certain extent [17]. These possible factors might have allowed sudden growth of micro-organisms in the periodontal pocket and resulted in positive BANA reaction. Thus, the ability of BANA to detect a particular threshold of anaerobic periodontopathic bacteria was found to be a valuable diagnostic tool for screening the individual at risk for an anaerobic infection and also as an objective indicator of periodontal disease activity that could be used in combination with the clinical criteria both to initiate therapy and as a means to monitor the efficacy of treatment [18].

Conclusion

From the observation of the study, following the conclusion was drawn suggesting that BANA-Enzymatic test™ may be a potential diagnostic tool, which could be employed. As a reliable indicator of BANA positive species in dental plaqueAs a simple, chair side test to detect a BANA hydrolyses from *P. gingivalis*, *T. denticola* and *T. forsythia*, anaerobic bacteria associated with adult periodontal diseaseAs an objective means of determining diseased sites, requiring some form of periodontal treatment. It

also helps in the different treatment options: Either the traditional approach of surgery or extraction of hopeless teeth or an approach based on an antimicrobial strategy.

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