Electronic Journal of Medical and Dental Studies
2022, 12(2), em0099
e-ISSN: 2753-2658
https://www.ejmds.com

MODESTUM

Original Article

OPEN ACCESS

Assessing periodontal health status of patients with chronic periodontitis before and after non-surgical therapy (SRP) using a BANA-Enzymatic™ test kit: A clinico-microbiological study

Tanusha Sharma [1]*, Charu Agarwal [2]

[1] Department of Periodontics, Ahmedabad Dental College & Hospital, Ahmedabad, Gujarat, INDIA
[2] Department of Periodontics, Goenka Research Institute of Dental Science, Gandhinagar, Gujarat, INDIA
***Corresponding Author:** drtanushas@gmail.com

Citation: Sharma T, Agarwal C. Assessing periodontal health status of patients with chronic periodontitis before and after non-surgical therapy (SRP) using a BANA-Enzymatic™ test kit: A clinico-microbiological study. ELECTR J MED DENT STUD. 2022;12(2):em0099. https://doi.org/10.29333/ejmds/12268

ARTICLE INFO

Received: 24 Jun. 2022

Accepted: 4 Jul. 2022

ABSTRACT

Background: There are many methods available for diagnosing and assessing periodontal health. Clinical improvements after non-surgical therapy (SRP) are directly linked to the microbiological changes, which indicates a decrease in certain periodontal microflora. The BANA-Enzymatic™ test kit is one of the modern and chair side alternatives to bacterial cultures. It detects the presence of three periodontal pathogens (*P. gingivalis, T. denticola,* and *T. forsythia*) known as the red complex microorganisms and thus serve as a marker of disease activity.

Aim and objective: The aim and objective of this study was to assess periodontal health status of patients with chronic periodontitis before and after SRP using a BANA-Enzymatic™ test kit.

Materials and methods: A total number of 20 patients comprising of both the sexes with chronic periodontitis were randomly selected for the present clinical study after meeting inclusion and exclusion criteria. Each selected sites were subjected to the assessment of plaque index, bleeding index, gingival index, pocket depth, and clinical attachment level before and after three months of SRP. BANA-Enzymatic™ test kit was used for the detection of micro-organisms. Statistical analyses were done.

Results: The results are statistically correlated to the BANA test and the clinical parameters that was recorded in the study. Hence, indicating the direct correlation with the severity of periodontal disease and destruction.

Conclusion: This study encourages a simple, easy, and chair side kit– BANA-Enzymatic™ test, as for diagnosis of periodontal condition. It is also helpful identifying the microbiological flora of the plaque (presence of red complex microorganisms).

Keywords: chronic periodontitis, non-surgical therapy, BANA-Enzymatic™ test

INTRODUCTION

Periodontal diseases, now recognized as bacterial infections, are among the most common, chronic diseases of humans, affecting 5% to 30% the adult population in the age group of 25 to *75* years. The pathogenic bacteria that grows in the oral biofilm, the gram negative anaerobic species developing in sub-gingival area are more aggressive for the periodontal structures [1]. Periodontal diseases result from an imbalance between the microbial flora inhabiting the periodontal pocket and the host's defense mechanisms against these pathogens. Destruction of periodontal tissues is a result of enzymatic activity present in the diseased area [2]. Sites with periodontitis have been shown to have greater proteolytic activity than those that are predominantly healthy. It has been suggested that there is a connection between the proteolytic activity of dental bacterial plaque and soft tissue destruction leading to chronic periodontitis [3].

However, bacterial culturing is expensive technique, which is highly sensitive and it is time taking whereas some organisms will not grow reliably on available culture media. Darkfield and phase contrast microscopic analysis can detect many of the microorganisms but they cannot fully specify these micro-organisms. Through microscopic evaluation one can detect mobile organisms but is not effective in identifying periodontal pathogens, which are non-motile. The cultural procedure has many methodological problems when used in periodontal microbiology [4]. There are additional method errors, with unknown error rates, that can be associated with the sampling procedure [5-8], the media used [7], the degree of anaerobiosis employed, and the type of dispersal procedures used [9]. The magnitude of these errors may vary with each of the cultivatable species found in the plaque [6] and could be as high as fivefold with some of the more fastidious species [7].

In contrast to the other detection methods the *B. forsythus*, *P. gingivalis*, and *T. denticola* isolates were confirmed by the BANA test (red complex microrganisms). The BANA test is developed by Walter J Loesche, which is very sensitive,

detecting small quantities of pathogens [8]. The BANA test is based on a modification of the BANA hydrolysis test, which in turn was an adaptation of the trypsin-like enzyme contained in the API-ZYM kit [8]. The anaerobic bacteria *porphyromonas (bacteroides) gingivalis* and/or *bacteroides forsythus* are unique in the subgingival flora in that they possess a trypsin-like enzyme which hydrolyzes the synthetic peptide benzoyl-DLarginine- naphthylamide or BANA.

The researchers in [8] proposed the use of BANA reaction to detect the presence of periodontal pathogens and thus serve as a marker of disease activity and also aid in monitoring periodontal therapy. Also it is a chairside kit with a sensitivity of 85%. The specificity of 53% would indicate that other BANA positive organisms are present in the plaque. Among the other species tested with the perioscan is B. forsythus, bacteroides and capnocytophaga species are able to give positive or weak reactions [8].

BANA is a rapid and reliable chair side diagnostic test, which can be performed in about 15 min time with unique ability of hydrolyzing the trypsin substrate, BANA for the red complex microrganisms.

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 chronic periodontitis patients.

MATERIALS AND METHODS

A prospective, interventional, microbiological study was planned on the population of individuals having chronic periodontitis. The study aimed in detection and comparing subjects with pre-treatment and post-treatment evaluations. A total number of 20 patients (80 sites) diagnosed with chronic periodontitis were selected [10]. Subjects in control group (group A) and 10 subjects in treatment group (group B). Both sexes were included aged 35-55 years and their daily routine, attitude and responsiveness were evaluated by asking routine questions. Subject who agreed to participate in the study and gave consent in front of witness, were ask to sign (thumb impression in case of illiterate subject) the written consent from along with the signature of the witness. The clinical study and samples were taken at baseline and after three months interval.

Inclusion Criteria

1. Subjects having good health.
2. Subjects with age group 35-55 years.
3. Subjects with definite clinical evidence of chronic periodontitis.
4. Subjects with minimum four sites of 5-7mm probing depth.
5. Male and female will be randomly selected.

Exclusion Criteria

1. Subjects having any history of systemic disease (cardiovascular disease, diabetes, hepatitis, and renal disorder).
2. Subjects having history of any local or systemic antimicrobial and anti-inflammatory therapy for last three months.

Figure 1. BANA-Enzymatic™ test kit

3. Subjects with a history of smoking or tobacco chewing.
4. Pregnant women or lactating women.
5. Inability of patient to co-operate because of their physical or mental status or daily routine.
6. Periodontal therapy other than standard prophylaxis during the previous three months.

Recording of Periodontal Parameters

Test was performed on four sites (upper and lower first molars) in each patients. Selected sites should have pocket≥ 5mm with adequate amount of subgingival plaque. Then clinical parameters were recorded and the plaque sample was collected at baseline for analysis of BANA micro-organisms. Then patient is subjected to scaling and root planing and recalled after three months and once again the clinical parameters and plaque samples were analyzed from the selected sites. Clinical periodontal parameters were recorded at baseline and three months following the periodontal treatment:

1. Plaque index [9].
2. Sulcus bleeding index [10].
3. Gingival index [10].
4. Probing pocket depth (with Williams graduated periodontal probe) [11].
5. Clinical attachment level [11].
6. BANA test (*N*-benzoyl-*d* L-arginine-2-napthylamide) [12].

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 and root planing using Gracey curettes performed under local anesthesia if required. Indices were recorded, BANA test was performed, and patient was recalled after three months. Clinical parameters and BANA test was again repeated after three months. **Figure 1** shows BANA enzymatic test kit.

Statistical Method

The statistical analysis was done using SPSS (statistical package for social sciences) version 16.0 statistical analysis software. The values were represented in number, percentage and mean±standard deviation. The correlation between the differences in mean values of PI, BI, GI, PPD, CAL, and BANA levels from baseline and three months after therapy was done by paired t-test.

Table 1. Comparison of mean values of plaque index at baseline and after three months

Particulars	Baseline (mean±SD)	After three months (mean±SD)	Mean difference (Mean±SD)	p-value	Interpretation
Plaque index	2.00±0.23	1.05±0.15	0.95±0.30	p<0.001	HS
Sulcus bleeding index	2.05±0.51	1.00±0.00	1.05±0.30	p<0.001	HS
Gingival index	2.01±0.45	1.07±0.18	0.94±0.27	p<0.001	HS
Periodontal pocket depth	6.05±0.28	4.50±0.28	1.55±0.41	p<0.001	HS
Clinical attachment level	5.97±0.88	4.57±0.54	1.04±0.15	p<0.001	HS

Table 2. Comparison of BANA-Enzymatic™ test scores at baseline and after three months

Particulars	Negative		Weakly positive		Positive	
	Number	Percentage	Number	Percentage	Number	Percentage
Baseline	0	00.0	6	7.5	74	92.5
After three months	28	35.0	40	50.0	68	85.0

Note. Chi-square=130.13, p<0.001 (highly significant)

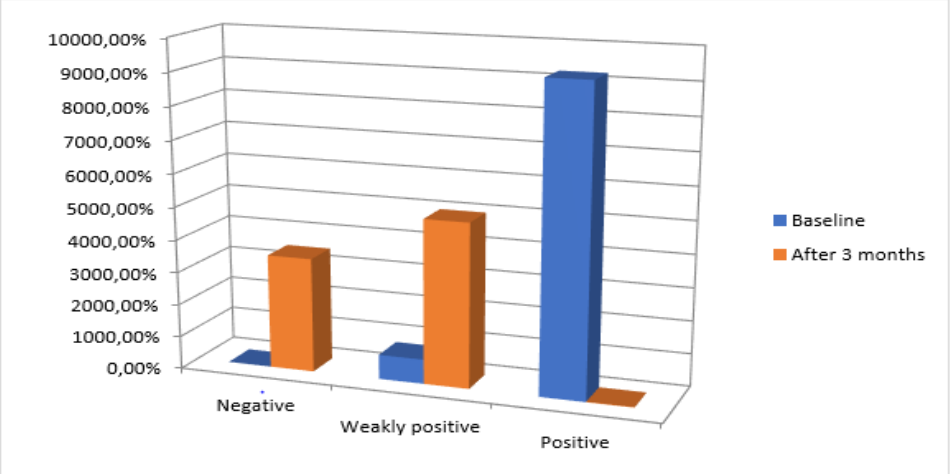

Figure 2. Comparison of BANA-Enzymatic™ test scores at baseline and after three months

The following formulas were employed for calculation of various parameters:

1. Mean/ average,
2. Standard deviation,
3. Paired student t- test,
4. Chi-square test,
5. Validity tests–sensitivity, specificity, and overall accuracy of test, and
6. Level of significance (p-value).

Comparison of different parameters of chronic periodontitis were done at baseline and after three months and their mean scores were compared by applying the paired t-test.

Plaque index, sulcus bleeding index, gingival index, periodontal pocket depth, and clinical attachment level were measured at baseline 2.00±0.23, 2.05±0.51, 2.01±0.45, 6.05±0.28, and 5.97±0.88, respectively and after three months, 1.05±0.15, 1.00±0.00, 1.07±0.18, 4.5±0.28, and 4.57±0.54 were as follows. The p- value evaluated p<0.001 and found to be highly significant (**Table 1**).

MICROBIOLOGICAL ANALYSIS

Comparison of BANA Test Scores at Baseline and After Three Months

Comparison of BANA test scores at baseline and after three months, which was analyzed using Chi-square test. The study includes 20 subjects with 80 sites.

At baseline

Analytic data of 0 site showed negative (0.0%), six sites showed weak positive, (7.5%), and 74 sites showed positive (92.5%).

After three months

Analytic data of 28 sites showed negative (35%), 40 sites showed weak positive (50%), and 68 sites showed positive (8.5%). The difference between the BANA-Enzymatic™ test results at baseline and after three months periodontal treatment was analyzed using the chi-square test and the results were found to be statistically highly significant (p<0.001). The results are shown in **Table 2** and **Figure 2**.

DISCUSSION

Periodontal disease is mainly due to gram-negative, anaerobic bacteria. These bacteria harm the host cells by producing variety of inflammatory markers which destruct the barrier and hence cause periodontal disease. Red complex microragnaism have a characteristic of trypsin like proteolytic activity. These are coomon to microrganisms like prophyromonus gingivalis, treponena denticola, and tannerella forsythia.

In [13], it was shown 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. A positive BANA test was more indicative of spirocheteal 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. In [14], the researchers agreed with the previous data. BANA test will not tell which of the organisms are present, but since they all are anaerobic species, it should enable the clinician to diagnose an anaerobic infection, and only such a diagnosis could be useful for the treatment and management of periodontal disease of the patient. These findings suggest that the BANA hydrolysis by the plaque has the potential to be an objective indicator of periodontal disease activity and could be used in combination with the clinical criteria both to initiate therapy and as a means to monitor the efficacy of treatment [13].

In this study the attempt is made to detect the levels of clinical and microbiological parameters before and after scaling and root planing by using BANA-Enzymatic™ test kit in chronic periodontitis. This study involved 20 patients with 80 sites having periodontal pockets, more than 5mm in depth. Scaling and root planing (SRP) results into the improvement in periodontal status of selected sites and were seen by the measurement of the periodontal status of the selected parameters and their correlation amongst pre and post operative treatment values. The mean values of clinical parameters reduced post-operatively which were statistically very highly significant ($p<0-001$). This results confirms the effectiveness of scaling and root planing as suggested in [15], that the elimination of supra and subgingival bacterial deposits can resolve inflammation and arrest disease progression. It is also supported in [16].

In present study the comparison between mean values of plaque index showed statistically high reduction after providing treatment to patients ($p<0.001$). This can be explained by the similar finding from the study of [12], who reported that there is reduction in the levels of spirochetes and black pigmented species after following non-surgical treatment. Hence, shown reduction in BANA positive plaque in the period immediately after treatment.

The BANA test is a simple, inexpensive chairside in-vitro test which can be used in the routine dental checkup. The test is designed to detect the presence of *treponema denticola, porphyromonas gingivalis,* and *bacteroides forsythus* in plaque samples. Hence by identifying these microrganisms one can go ahead with the treatment plan and choice of antibiotics. The BANA hydrolysis test kit is highly sensitive which has got limitations–firstly test strips can be used only one time. Secondly, it can detect red complex microrganisms but cannot detect individual microrganisms. Thirdly, blood and too little plaque or too much plaque can give a false negative result. Also, there can be error in identifying and judging the results.

CONCLUSION

Therefore, we can conclude that non-surgical periodontal therapy includes scaling and root planning and detection of red complex microorganisms by BANA test is an essential component for detecting and evaluating the outcome of chronic periodontitis. So, this clinical study concludes that BANA test using subgingival plaque sample may be a potential diagnostic tool. It is a reliable indicator of red complex microorganisms in plaque. It is also a simple, easy and quick method for identifying the diseased sites, these sites require treatment. It can determine the need for retreatment. Hence this type of diagnostic method is replacing the old traditional methods with accurate and rapid chairside detection of microorganisms.

Author contributions: All authors have sufficiently contributed to the study, and agreed with the results and conclusions.

Funding: No funding source is reported for this study.

Declaration of interest: No conflict of interest is declared by authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

REFERENCES

1. Haffajee AD, Socransky SS. Microbiology of periodontal diseases. Periodontol 2000. 2005;38:9-12. doi:10.1111/j.1600-0757.2005.00112.x PMid:15853934
2. Beighton D., Radford JR, Naylor MN. Protease activity in gingival crevicular fluid from discrete periodontal sites in humans with periodontitis or gingivitis. Arch Oral Biol. 1970;35:329-35. doi:10.1016/0003-9969(90)90179-E
3. Frostel G, Soder PO. The proteolytic activity of plaque and its relation to soft tissue pathology. Int Dent J. 1970;29:436-50.
4. Genco RJ, Goldman H, Cohen DW. Pathogenesis and host response in periodontal disease. Contemp Periodontics. 1990;184-93.
5. Dahlen G, Renvert S, Wickstrom M, Egelberg J. Reproducibility of microbiological samples from periodontal pockets. J Clin Periodontol. 1990;17:73-7. doi:10.1111/j.1600-051X.1990.tb01065.x PMid:2406295
6. Mombeili A, Minder CE, Gusberti FA, Lang NP. Reproducibility of microscopic and cultural data in repeated subgingival plaque samples. J Clin Periodontol. 1989;16:434-42. doi:10.1111/j.1600-051X.1989.tb01672.x PMid:2768538
7. Moore WEC. Microbiology of periodontal disease. J Periodontal Res. 1987;22:335-41. doi:10.1111/j.1600-0765.1987.tb01595.x PMid:2961863
8. Loesche WJ, Bretz WA, Kerschensteiner D et al. Development of a diagnostic test for anaerobic periodontal infections based on plaque hydrolysis of benzoyl-DL-arginine-naphthylamide. J Clin Microbiol. 1990;1551-9. doi:10.1128/jcm.28.7.1551-1559.1990 PMid:2380379 PMCid:PMC267987
9. Silness J, Loe H. Periodontal disease in pregnancy. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand. 1964;22:121-35. doi:10.3109/00016356408993968 PMid:14158464

10. Muhlemann HR, Son S. Gingival sulcus bleeding: A leading symptom in initial gingivitis. Helv Odontol Acta. 1971;15:107.
11. Fleming I, Thorkild K, Rolf A. Reproducibility of pocket depth and attachment level measurements when using a flexible splint. J Clin Periodontol. 1984;11:662-8. doi:10.1111/j.1600-051X.1984.tb01314.x PMid:6594353
12. Loesche WJ, Syed SA, Stoll J. Trypsin-like activity in subgingival plaque. A diagnostic marker for spirochetes and periodontal disease. J Periodontol. 1987;58:266-73. doi:10.1902/jop.1987.58.4.266 PMid:3473222
13. Loesche WJ, Taylor GW, Giordano J, et al. A logistic regression model for the decision to perform access surgery. J Clin Periodontol. 1997;24:171-9. doi:10.1111/j.1600-051X.1997.tb00487.x PMid:9083901
14. Bretz WA, Loesche WJ. Characteristics of trypsin-like reaction in subgingival plaque samples. J Dent Res. 1987;66:1668-72. doi:10.1177/00220345870660111301 PMid:10872404
15. Gray G. Periodontal response to mechanical non-surgical therapy. A review. J Periodontol. 1992;63:118-30. doi:10.1902/jop.1992.63.2.118 PMid:1552465
16. Kleber BM. First results with a microbiological quick diagnostic procedure in periodontitis. Dtsch Stomatol. 1991;41(12):460-2.