

Assessment of efficacy of chlorhexidine chip as an adjunct to scaling and root planning using N-benzoyl-DL-arginine-2-naphthylamide test kit

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ABSTRACT

Background: With increasing advances in the field of medicine, diagnosing a disease has been an easy task and periodontitis is no exception to this. N-benzoyl-DL-arginine-2-naphthylamide (BANA) test is a modern chair-side paraclinical method designed to detect the presence of one or more anaerobic bacteria commonly associated with periodontal disease, namely *Treponema denticola*, *Porphyromonas gingivalis*, and *Tannerella forsythia* in subgingival plaque samples taken from periodontally diseased teeth. **Aim:** The aim of the study was to assess the efficacy of chlorhexidine chip as an adjunct to scaling and root planning (SRP), using BANA Test Kit. **Materials and Methods:** A total of 20 chronic periodontitis patients (aged 35–55 years) having pocket depth of ≥ 5 mm in molar teeth were selected and randomly divided into following treatment groups: Group I: SRP and Group II: SRP along with chlorhexidine chip. The clinical and microbial parameters were recorded at baseline and 1 and 3 months post-treatment. BANA chairside test was used for estimation of specific microbiota. Statistical analysis used: Mann-Whitney test, Wilcoxon signed test, *t*-test, Pearson's Chi-square test, and variability test were used. **Results:** Plaque index, modified bleeding index, probing pocket depth, and clinical attachment level scores in selected teeth within the groups at different time intervals were highly significant ($P < 0.001$) after the 3rd month. Although the comparison between groups for specific microbiota in selected sites at different intervals was not statistically significant at baseline and 1 month, it reached statistical significance at the 3rd-month post-treatment, and significant reductions in percentage of BANA positive sites were observed in both groups, Group II had significantly greater percentage of BANA negative sites. **Conclusion:** Local drug delivery using chlorhexidine chip enhances the benefit of SRP in the treatment of chronic periodontitis.

Key words: N-Benzoyl-DL-arginine-2 naphthylamide, chlorhexidine, chronic periodontitis, local drug delivery

INTRODUCTION

Oral cavity offers diverse habitats wherein different species of microorganisms can prosper and an aggregation of which is referred to as dental plaque. Microbes in dental plaque flourish in niche to adhere to the tooth surfaces and multiply in shielded environment such as periodontal pockets and tooth crevices. In 1998, Socransky *et al.* suggested that most of the pathogenic of all complexes comprised *Porphyromonas gingivalis* (Pg), *Tannerella forsythia*, and *Treponema denticola* (Td) also collectively known as "Red Complex." These red complex bacteria have shown to be most important periodontal pathogens in causing periodontal disease.^[1] These pathogens produce various trypsin-like enzymes that degrade intercellular matrix of periodontal tissues.^[2] Since the 1990s, there has been the emergence of a multitude of diagnostic tests based on microbiological, chemical, physical, and immunological methodologies. However, bacteria culture is difficult and often requires more training than molecular technique,^[3] and some organisms do not grow reliably on available culture media. Darkfield and phase contrast microscope analysis can detect a number of microorganisms and their morphotypes,

but have limited application due to their inability to fully specify these microorganisms. Microscopic evaluation can also be helpful in detecting mobile organisms but is not effective in identifying periodontal pathogens, which are non-motile.^[4] Until date, the diagnostic tools are based on enzymes diagnostic markers and DNA probes to identify specific periodontopathogenic bacteria, so as to enforce preventive and therapeutic measures toward disease control. N-benzoyl-DL-arginine-2-naphthylamide (BANA) Enzymatic test™ (OraTec Corporation, Manassas, USA) is a rapid and reliable chairside diagnostic test, which can be performed in about 15 min time and can give information about the presence of three of the putative pathogens in subgingival plaque samples. Walter Loesche *et al.* studied a strong relationship between a BANA positive reaction and high levels of plaque spirochetes and proposed BANA reaction to detect the presence of periodontal pathogens and thus, serving it as a marker for disease activity and also for monitoring the periodontopathogenic.^[5]

The therapeutic approach for periodontitis is to remove the bacteria, either with hand instrumentation or with electronic instrumentation. Another approach is to use chemotherapeutic

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agents systemically or locally to limit the bacteria. The systemic antibiotic therapy has various disadvantages such as hypersensitivity reactions, organ toxicity, and development of resistant bacteria.^[6] There are various local drug delivery systems available, but since long, chlorhexidine has been one of the most effective topical antimicrobial agents and is on the World Health Organization's list of essential medicines.^[7] It is used as a controlled subgingival local drug delivery. The bactericidal effect of the drug is due to the cationic molecule binding to extra microbial complex and negatively charged microbial cell walls, thereby altering the osmotic equilibrium of cells. It also inhibits plaque formation by binding to anionic groups on salivary glycoproteins thus, reducing pellicle formation. The main advantage of using chlorhexidine chip over mouthwash is that it allows sustained release of this agent at the infection site thus, prolonging its bactericidal effect.

STUDY AND METHODS

The present study was carried out in the Department of Periodontology and Oral Implantology in our institution from moderate to severe periodontitis of an age group of 35–55 years and having bilateral pockets of 5–7 mm in molars were selected for the study. However, patients having any systemic disease or undergoing any local or systemic antimicrobial and anti-inflammatory therapy for past 6 months or periodontal therapy other than standard prophylaxis during previous 6 months were excluded from the study. Pregnant, lactating women, tobacco chewers, and alcoholics were also excluded from the study and the study was approved from Institutional Ethical Committee before the start of the study.

Study Design

A 3 months simple randomized, clinical study was conducted comparing the effect of SRP with and without chlorhexidine chip in chronic periodontitis patients. A total of 20 patients with 40 sites were randomly divided into following two groups:

- i. Group I: Patients treated with scaling and root planning (SRP).
- ii. Group II: Patients treated with SRP along with placement of chlorhexidine chip.

The nature and design of the clinical study were explained, and informed consent was obtained from all the participants. The clinical parameters recorded in the pro forma included plaque index (PI), modified bleeding index (m-BI), probing pocket depth (PPD), and clinical attachment levels (CAL).

One molar site on each arch with pocket depth of ≥ 5 mm was selected in each patient for the study, and SRP was performed for both group. The subgingival placement of chlorhexidine chip was done after proper isolation of the area in Group II. All patients were given oral hygiene instructions. Microbiological study BANA chair side test which is a modification of BANA hydrolysis was used to estimate specific microbiota.

Working Principle of BANA

An unusual enzyme produced by specific bacteria Td, Pg, or *Tannerella forsythia* (Tf) capable of hydrolyzing the synthetic peptide BANA present on BANA test strips, in turn, reacts with embedded diazo dye to produce the permanent blue color indicating a positive test.

Procedure

Subgingival plaque sample was obtained using curette and applied on to the raised reagent matrix affixed to the lower portion of the test strip. The upper test strip was moistened (salmon color) with distilled water using a cotton swab. Care was taken not to over wet. BANA-zyme test strip was folded at the given crease mark so that the lower and upper reagent strips were meet.

BANA-zyme test strip was placed into either of the slots on the top of the processor. The heating element of the processor started automatically when strip was inserted into the bottom of the slot, as indicated by the flashing light. When the indicator light remained on, it meant the heating element had reached 55°C. The BANA-zyme test strips color development was completed when the indicator light went off and the bell rang. BANA-zyme strip was removed from the processor, and the lower reagent strip was discarded that had been inoculated with plaque in a manner appropriate for contaminated material. The upper reagent strip was examined for the presence of blue color. If a blue color was detected, the site was marked as either weak positive or positive.

Recording was done for each sampled site as negative, weak positive, or positive.

RESULTS

All patients (15 males and 5 females with mean age of 35 ± 5 years) completed the study. Both clinical and microbiological recordings were carried out at baseline and 1 and 3 months post-treatment. All recordings were subjected for statistical analysis using Mann-Whitney, Wilcoxon test, *t*-test, Pearson Chi-square test, and variability test.

PI, m-BI, PPD, and CALs scores between both the groups were similar at baseline and 1st month. However, there was a significant reduction in pocket depth and gain in CALs in Group II as compared to Group I at the end of the 3rd month [Table 1]. Microbiological assessment between the groups at different intervals showed no significance at baseline and 1 month; however, statistically significant difference was observed at the 3rd-month post-treatment. Although significant reduction in percentage of BANA positive sites was observed in all the three groups, Group 2 showed significantly greater percentage of BANA negative sites [Table 2]. Variability was performed for both groups [Table 3] and it showed that BANA is sensitive but has low specificity.

DISCUSSION

Periodontal disease is a polymicrobial infection primarily caused by periodontal pathogens existing within the subgingival plaque. BANA Test was used to detect the presence of periodontal pathogens which served as a reliable marker of disease activity and also aided in monitoring periodontal therapy. The conventional method of lowering the bacterial load in the periodontal pocket constitutes SRP, but to prevent recolonization the use of adjunctive methods has been advocated. The use of controlled release devices enables maintenance of concentration of antimicrobial agent within the pocket.

The study was conducted to evaluate the clinical efficiency of chlorhexidine chip and assess the specific microbial changes

Table 1: Comparison of all clinical parameters for Group I and Group II at baseline, 1st month and 3rd month

Parameters	Baseline, M±SD		1 st month, M±SD		3 rd month, M±SD	
	Group I	Group II	Group I	Group II	Group I	Group II
Plaque index	2.52±0.40	2.58±0.39	1.56±0.41	1.30±0.35	1.06±0.37	0.68±0.26
Significance (P value)	0.608		0.039*		0.001**	
Modified bleeding index	2.78±0.40	2.98±0.17	1.45±0.27	1.16±0.39	0.47±0.31	0.61±0.29
Significance (P value)	0.076		0.017*		0.001**	
Pocket depth	6.60±0.88	7.00±0.72	5.35±0.87	5.15±0.48	4.90±0.64	4.25±0.55
Significance (P value)	0.126		0.378		0.001**	
Clinical attachment level	7.25±0.96	7.75±0.85	6.15±1.08	5.80±0.83	5.65±0.93	4.75±0.63
Significance (P value)	0.091		0.261		0.001**	

Mann-Whitney test, Wilcoxon test, and t-test were performed for intergroup comparison for mean plaque index, modified bleeding index, probing depth and clinical attachment levels in Group I and Group II at baseline, 1st month and 3rd month and these values were found to be statistically significant ($P < 0.05$) at 3rd month in both Group I and Group II. *P value significant. **P value highly significant. M: Mean, SD: Standard deviation

Table 2: Color change in BANA test at baseline 1st month and 3rd month in Group I and Group II

Parameters	Baseline			1 st month			3 rd month			Significance (Pearson's Chi-square test)
	P	WP	N	P	WP	N	P	WP	N	
Group I	13	7	0	1	17	2	0	15	4	0.000**
Group II	16	4	0	0	17	3	0	04	16	0.000**

Pearson's Chi-square test was performed for intergroup comparison for color change in BANA test in Group I and Group II at baseline, 1st month and 3rd month. Color change after BANA test at 3rd month was statistically significant ($P < 0.05$) in both Group I and Group II. Where: P: Positive color change, WP: Weak positive color change, and N: Negative color change. **P value highly significant. BANA: N-benzoyl-DL-arginine-2-naphthylamide

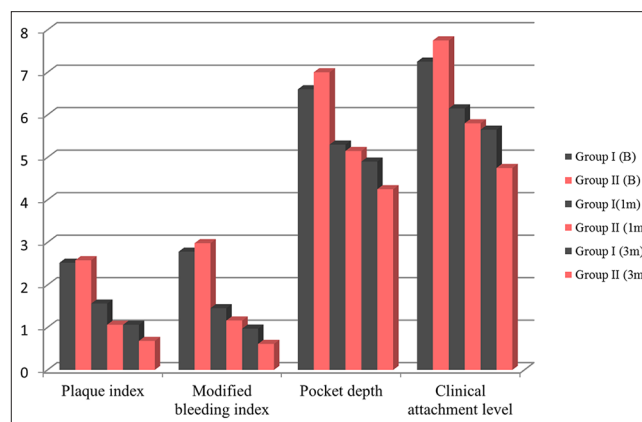
Table 3: Validity test for Group I and Group II

Parameters	Sensitivity		Specificity	
PI	93.33%	73.68%	33.33%	33.33%
m-BI	94.73%	88.88%	33.33%	33.33%
Probing periodontal depth	86.66%	98.33%	66.66%	66.66%
CAL	98.33%	88.33%	32.42%	44.58%
BANA test	68.15%	63.08%	18.85%	41.66%

Validity test was performed for both Group I and Group II. It showed that BANA test was highly sensitive but low in specificity. PI: Plaque index, m-BI: Modified bleeding index, CAL: Clinical attachment level, BANA: N-benzoyl-DL-arginine-2-naphthylamide

associated with chronic periodontitis patients. The study was conducted for 3 months period because, effects of controlled release chlorhexidine have been shown to be evident up to 11 weeks after administration of the chip and 3 months study period corresponds to typical recall interval for periodontal patients.^[8,9]

All patients showed statistically and clinically significant improvements in PI at the follow-up visits when compared to the baseline level. Table 1 and Graph 1 show that the PI of Group I changed from 2.52 ± 0.40 at baseline to 1.56 ± 0.41 in 1st month and to 1.06 ± 0.37 in 3rd month. For Group II, it changed from 2.58 ± 0.39 at baseline to 1.30 ± 0.35 in 1st month and to 0.68 ± 0.26 in 3rd month. The reduction in PI could be due to proper oral hygiene maintenance and thoroughness of SRP. The reduction in PI in Group II was significantly more when compared to Group I. Similar reduction in PI between chlorhexidine group and SRP group has been shown in the study conducted by Puri *et al.*^[10] The

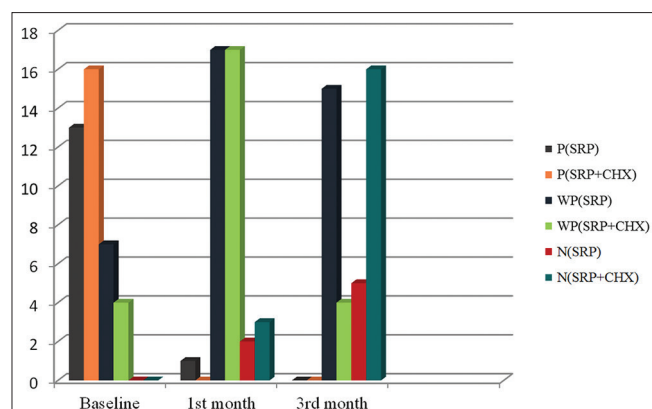


Graph 1: Comparisons of clinical parameters in Group I and Group II at baseline, 1st month, and 3rd month. Graph 1 shows the comparisons of clinical parameters at baseline, 1st month and 3rd month between Group I and Group II. X-axis shows clinical parameters at baseline, 1st month and 3rd month and Y-axis shows mean values of plaque index (PI), modified bleeding index (m-BI), periodontal pocket depth (PPD), and clinical attachment level (CAL) for Group I (scaling and root planning [SRP]) and Group II (SRP + CHX)

reduction in PI for chlorhexidine group is attributed to the fact that the 2.5 mm chip delays reproduction of bacteria by inhibiting their proteolytic and glycosidic activities.^[11]

The bleeding index scores also reduced in both groups. Bleeding index scores of Group I changed from 2.78 ± 0.40 at baseline to 1.45 ± 0.27 in 1st month and to 0.97 ± 0.97 in 3rd month. For Group II, it changed from 2.98 ± 0.17 at baseline to 1.62 ± 0.39 in 1st month and to 0.61 ± 0.29 in 3rd month and is shown in Table 1 and Graph 1. The reduction in bleeding scores in Group II was significantly more than Group I. Similar results of reduction in bleeding index between chlorhexidine group and SRP group has been shown in study conducted by Paolantonio *et al.*^[12] The reduction in bleeding on probing could be attributed to the elimination of local factors with SRP in Group I and II, respectively, which is in conjunction with study conducted by Carvalho *et al.*^[9]

Increased probing depth and loss of clinical attachment loss are pathognomonic for periodontitis and hence pocket probing is crucial and mandatory procedure in diagnosing periodontitis and evaluating the success of periodontal therapy. The probing



Graph 2: Color change in N-benzoyl-DL-arginine-2-naphthylamide (BANA) test for Group I and Group II at baseline, 1st month, and 3rd month. Graph 2 shows the comparisons of color change in BANA test in Group I (scaling and root planning [SRP]) and Group II (SRP + CHX). X-axis shows color change at baseline, 1st month, and 3rd month and Y-axis shows mean values of color change. Where: P: Positive, WP: Weak Positive and N: Negative

depth also reduced in both groups. Probing depth of Group I changed from 6.60 ± 0.83 at baseline to 5.35 ± 0.87 in 1st month and to 4.90 ± 0.64 in 3rd month. For Group II, it changed from 7.00 ± 0.72 at baseline to 5.15 ± 0.48 at 1st month and to 4.25 ± 0.55 in 3rd month and this was shown in Table 1 and Graph 1. This was in accordance with the study conducted by Sosklone *et al.*^[13] The reduction in pocket depth could be attributed to soft tissue shrinkage following SRP as well as resolution of gingival inflammation due to antimicrobial agent.^[14]

The CAL also reduced in both groups. CAL of Group I changed from 7.25 ± 0.96 at baseline to 6.15 ± 1.08 in 1st month and to 5.65 ± 0.93 in 3rd month. For Group II, it changed from 7.75 ± 0.85 at baseline to 5.80 ± 0.83 at 1st month and to 4.75 ± 0.63 in 3rd month. This was shown in Table 1 and Graph 1 and was in accordance with the study conducted by Rodrigues *et al.*^[15] The greater gain in CAL in Group II could be attributed to the absence of bacterial challenge, caused by retained antimicrobial agent during critical initial phase of healing following SRP.^[16]

The microbiological assessment was performed using a chairside BANA test showing specific microbiota. Tf, Pg, Td, and *Capnocytophaga* species share a common enzymatic profile and have a trypsin-like enzyme in common. The activity of this enzyme can be measured with the hydrolysis of the colorless substrate BANA; hence, all the three BANA positive species are frequently cited as potential periodontal pathogens.^[17] In our study, the percentage of BANA positive sites for the detection of a number of putative periodontal bacteria was reduced significantly from baseline to 1 month and 3 months within the groups. In Group 2, the number of BANA negative sites was comparatively higher than the Group I. This could be due to more effective control of the periodontal anaerobic microorganisms enhancing the effect of SRP. This is in accordance with previous studies.^[8,13] When BANA test sites were compared between the groups, it did not show significance at 1 month; however, significant differences were observed at 3 months after treatment with Group II better over Group I and this was shown in Figures 1-3, Table 2, and Graph 2. These results explain that SRP alone will reduce the bacterial



Figure 1: Color change in N-benzoyl-DL-arginine-2-naphthylamide strip at baseline

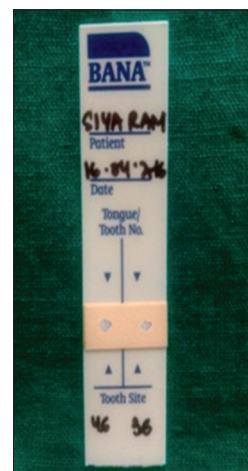


Figure 2: Color change in N-benzoyl-DL-arginine-2-naphthylamide strip at the 1st month



Figure 3: Color change in N-benzoyl-DL-arginine-2-naphthylamide strip at the 3rd month

load at 1 month, but additional benefits are observed with chlorhexidine chip at 3 months in reducing the microbial load.

Validity test was performed for all parameters for both Group I and Group II. In Group I and II, sensitivity values for PI were 93.33% and 73.68%, for m-BI was 94.73% and 88.88%, for pocket depth was 86.66% and 98.33%, for CAL was 98.33% and 88.88%, and for BANA test was 68.15% and 63.08%, respectively. Similarly, specificity values for PI were 33.33% and 33.33%, for m-BI was 33.33% and 33.33%, for pocket depth was 66.66% and 66.66%, for CAL was 32.42% and 44.58%, and for BANA test was 18.85% and 41.66%, respectively. These were in correlation with study conducted by Andrade *et al.*^[3] this was shown in Table 3. This clearly showed that BANA test was more sensitive but was low in specificity as it could only determine the presence of red complex bacteria but could not specify the type of bacteria.

CONCLUSION

BANA test can be used as a reliable indicator of BANA positive species in dental plaque and as an objective means of determining diseased sites, requiring some form of periodontal treatment.

No adverse events were reported with the use of chlorhexidine chip; hence, it is safe and effective when used as an adjunct to SRP in the treatment of periodontitis.

REFERENCES

- Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. *J Clin Periodontol* 1998;25:134-44.
- Slots J, Bragd L, Wikstrom M, Dahlen G. The occurrence of *Actinobacillus actinomycetemcomitans*, *Bacteroides gingivalis* and *Bacteroides intermedius* in destructive periodontal disease in adults. *J Clin Periodontol* 1986;13:570-7.
- Andrade JA, Feres M, Figueiredo LC, Salvador SL, Cortelli SC. The ability of the BANA test to detect different levels of *P. gingivalis*, *T. denticola* and *T. forsythia*. *Braz Oral Res* 2010;24:224-30.
- Genco RJ, Goldman H, Cohen DW. Contemporary Periodontics. St. Louis: The CV Mosby Company; 1990.
- Loesche WJ, Lopatin DE, Giordano J, Alcoforado G, Hujel P. BANA as accurate as DNA testing for periodontal pathogens. *J Clin Microbiol* 1992;30:427-33.
- Jain M, Dave D, Jain P, Manohar B, Yadav B, Shetty N. Efficacy of xanthan based chlorhexidine gel as an adjunct to scaling and root planing in treatment of the chronic periodontitis. *J Ind Soc Periodontol* 2013;17:439-43.
- Mundinamane DB, Venkataraghavan K, Garg A. Newer trends in local drug delivery for periodontal problems—A preview. *Int J Contemp Dent* 2011;2:8-10.
- Grisi DC, Salvador SL, Figueiredo LC, Souza SL, Novaes AB, Grisi MF. Effect of a controlled-release chlorhexidine chip on clinical and microbiological parameters of periodontal syndrome. *J Clin Periodontol* 2002;29:875-81.
- Carvalho J, Novak MJ, Mota LF. Evaluation of the effect of subgingival placement of chlorhexidine chips as an adjunct to scaling and root planing. *J Periodontol* 2007;78:997-1001.
- Puri K, Dodwad V, Bhat K, Puri N. Effect of controlled-release Periochip™ on clinical and microbiological parameters in patients of chronic periodontitis. *J Ind Soc Periodontol* 2013;17:605-11.
- Jones CG. Chlorhexidine. Is it still the gold standard? *Periodontol* 2000 1997;15:55-62.
- Paolantonio M, D'Angelo M, Grassi RF, Perinetti G, Piccolomini R, Pizzo G, *et al.* Clinical and microbiologic effects of subgingival controlled-release delivery of chlorhexidine chip in the treatment of periodontitis: A multicenter study. *J Periodontol* 2008;79:271-82.
- Soskolne WA, Heasman PA, Stabholz A, Smart GJ, Palmer M, Flashner M, *et al.* Sustained local delivery of chlorhexidine in the treatment of periodontitis: A multi-center study. *J Periodontol* 1997;68:32-8.
- Soskolne WA, Proskin HM, Stabholz A. Probing depth changes following 2 years of periodontal maintenance therapy including adjunctive controlled release of chlorhexidine. *J Periodontol* 2003;74:420-7.
- Rodrigues IF, Machion L, Casati MZ, Nociti FH Jr. de Toledo S, Sallum AW, *et al.* Clinical evaluation of the use of locally delivered chlorhexidine in periodontal maintenance therapy. *J Periodontol* 2007;78:624-8.
- Paul GT, Hemalata M, Faizuddin M. Modified widman flap and non-surgical therapy using chlorhexidine chip in the treatment of moderate to deep periodontal pockets: A comparative study. *J Indian Soc Periodontol* 2010;14:252-6.
- Amalfitano J, De Filippo AB, Bretz WA, Loesche WJ. The effects of incubation length and temperature on the specificity and sensitivity of the BANA (N-benzoyl-DL-arginine-naphthylamide) test. *J Periodontol* 1993;64:848-52.

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