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Research Article

- BANA Hydrolysis as a Comparative Tool in the Evaluation of Amoxicillin and Azithromycin in the Treatment of Chronic Periodontitis

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Abstract

Background and aims. The present study was envisaged to compare the efficacy of adjunctive use of Azithromycin with scaling and root planing (SRP) the adjunctive use Amoxicillin and SRP, and SRP alone in the treatment of chronic periodontitis. To correlate the use of N-Benzoyl DL-Arginine 2- Naphthylamide (BANA) hydrolysis test and percentage of spirochete count with the periodontal parameters before and after periodontal therapy.

Materials and methods. Thirty subjects with chronic periodontitis were randomly selected and divided into three groups as follows. Group I: Subjects treated with scaling and root planing (SRP) alone. Group II: Subjects treated with SRP and systemic administration of Amoxicillin (SRP + AMOX). Group III: Subjects treated with SRP and systemic administration of Azithromycin (SRP + AZM). Periodontal parameters comprising of plaque index, bleeding index, probing pocket depth, clinical attachment level and microbiological parameters comprising of spirochete count and BANA test scores were assessed at base line and six weeks after completion of periodontal therapy for subjects in all the three groups.

Results. The reduction in post-treatment scores as compared to pre-treatment scores of plaque index, bleeding index, pocket depth, clinical attachment levels and spirochete count was highly significant in all the groups($p<0.01$).BANA hydrolysis is a reliable marker of periodontal disease as it has proved to be a suitable test for detection of spirochetes.

Conclusion. The judicious use of systemic antibiotics in the treatment of chronic periodontitis may provide an additional benefit in the clinical outcome compared to SRP alone.

Key words: **Chronic periodontitis, BANA hydrolysis test, amoxicillin and azithromycin, scaling and root planing.**

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.[1] These are multi factorial infections, elicited by a complex of bacterial species, that interact with the host tissue cells, and release an array of cytokines, chemokines and mediators leading to the destruction of periodontal supporting structures.[2]

The disease progresses in bursts of activity and exists in active and inactive states if not treated.[3] Though chronic periodontitis has a bacterial etiological factor, it is also influenced by smoking, education, social and economic status, age, pregnancy, genetics and systemic diseases.[4] A variable susceptibility is seen depending on the host response to periodontal pathogens.[5] In order to control the progress of chronic periodontitis, early diagnosis is very crucial, though periodontal pocket does not give idea of disease progression, it should be noted that pocket depth provides a historical record of the disease.

Studies by some authors have classified microbiota into groups or complexes and proposed that the red complex of microorganisms consists of Porphorymonous gingivalis (PG), Tannerella forsythensis (TF) and Treponema denticola (TD) .[6] These red complex species possess unique properties which include trypsin like activity usually detected in sub gingival plaque samples when the bacterial level is high. The BANA test is quite effective for the detection of red complex organisms and is useful in the initial diagnosis of chronic periodontitis. This has led to the development of certain indicators for the identification of disease sites using phase contrast or dark field microscopy, bacterial culture, immunoassays, nucleoid probes enzyme assays and polymerase chain reaction assays and in addition the BANA hydrolysis test.[7]

To correlate the use of BANA hydrolysis test with the periodontal parameters before and after periodontal therapy Loesche 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[8]. Microbiological and immunological factors have been found to be responsible for the pathology of periodontal disease.[9,10] Hence, this study was aimed to compare the clinical and bacteriological changes occurring in the 3 groups of chronic periodontitis patients before and after treatment using BANA as a marker of periodontal disease. To compare the antimicrobial efficacy of adjunctive use of Azithromycin with SRP, the adjunctive use of Amoxicillin and SRP and the use of SRP alone in the treatment of chronic periodontitis.[11,12,13]

Materials and Methods

A total number of 30 subjects were selected with chronic periodontitis from the department of periodontics, Rama Dental College & Hospital. Consent was obtained prior to participation.

Inclusion Criteria:

1. Subjects in the age group of 25-55 years having at least 24 natural teeth and at least 1 molar tooth in each quadrant of the mouth.
2. Subjects who were free from relevant allergies and systemic diseases.
3. Subjects who had not received any surgical/non-surgical periodontal therapy or any antibiotic therapy for past 6 months.
4. Subjects who were willing to attend the hospital at frequent intervals.

Subjects were diagnosed with chronic generalized periodontitis when atleast 8 sites with a probing depth >5mm and attachment loss greater than 2 mm were present. The selected subjects were randomly assigned to one of the three groups based on the treatment method.

Group I (SRP alone) n=10, scaling and root planing without any systemic antibiotics.

Group II (SRP+AMOX) n=10, scaling and root planing with systemic administration of Amoxicillin 250 mg TID for 7 days.

Group III (SRP+AZM) n=10, scaling and root planing along with systemic administration of Azithromycin500 mg, OD for 3days.

Clinical Assessments: The following clinical parameters were recorded for subjects in all the three groups.

1. Plaque index
2. Bleeding Index
3. Probing pocket depth
4. Clinical attachment level

Microbiological Examination-Sample Collection -

Sub gingival plaque was collected from 4-6 most diseased tooth sites using a sterile curette. Thereafter, the curette tip was vigorously agitated in a test tube containing 0.6 ml of Sorensen buffer solution at pH of 7.2, and placed for 20 s in a vortex mixer to get a homogenous plaque suspension.[14]

Dark Field Microscopic Examination: A 10 L of plaque suspension was placed on to a glass slide and examined under10 x magnification of dark field microscope for evaluation of spirochetes.

Enzyme Assay (BANA HydrolysisTest)

The plaque sample was added to the working solution of BANA a drop of fast black potassium salt diazonium reagent was added and the degree of blue-black color was assessed after the BANA hydrolysis test , visually and scored using the following scale.

Negative = no color beyond that of salmon color of the dye.

Positive = a faint blue-black to distinct blue-black color. [15,16]

The result of the test in each subject was noted and recorded.

Statistical Analysis

The results of the study were subjected to statistical analysis by ANOVA and Pearsons correlation coefficient. p <0.01 values were considered highly significant.

Results

Plaque index, bleeding index, probing pocket depth (PPD) and clinical attachment levels were assessed at base line and 6 weeks after the completion of periodontal therapy for all the subjects.

Table 1 reveals that there was no significant difference in the mean pocket depths between the groups I, II and III at baseline. There was statistically highly significant difference between pre and post treatment scores within the groups (p<0.01).

Clinical attachment level CAL): There was no significant difference in the mean scores of groups I, II and III at baseline. There was statistically highly significant difference between pre and post treatment scores within the groups (p<0.01).

BANA Assay: No significant difference in the mean BANA scores of the three groups at baseline was seen. There was statistically highly significant difference between pre and post treatment scores within the groups (p<0.01).

Spirochete count: No significant difference was found in the mean spirochete count of the three groups at baseline. Highly significant difference was found within the groups after the treatment (table 4).

Correlation between different variables

A negative correlation co-efficient was found between plaque index and spirochete count and between plaque index and BANA scores for Groups I, II and III combined after treatment.

A positive correlation coefficient was found between bleeding on probing and spirochetes count, between BANA and spirochete count, between pocket depth and spirochete count and between clinical attachment level and spirochete count for groups I,II and III combined after treatment. This was found to be statistically significant (p<0.05).

A positive correlation coefficient was found between bleeding index and BANA scores, between probing pocket depth and BANA scores, between CAL and BANA scores, between spirochete count and BANA scores.

These results suggest that to control periodontal disease, it is necessary to improve and control clinical parameters such as the bleeding index, probing pocket depth and CAL.

Table 1. Comparison of mean Probing Pocket depth (mm) within the group before and after treatment

Group	Period	Mean	SD	Mean Change	Paired t-value	P-value	Sig
I	BL	5.02	0.419	1.50	9.65*	0.000	S
	6 W	3.52	0.80				
II	BL	5.03	0.51	1.85	19.89*	0.00	S
	6 W	3.17	0.41				
III	BL	5.10	0.57	1.76	9.47*	0.000	S
	6 W	3.34	0.80				

The mean difference is significant at the 0.01 level (p<0.001)

Table 2. Comparison of mean CAL (mm) within the group before and after treatment

Group	Period	Mean	SD	Mean Change	Paired t-value	P-value	Sig
I	BL	3.027	0.742	1.30	8.158	0.00	S
	6 W	1.724	0.684				
II	BL	3.046	0.423	1.86	18.239	0.00	S
	6 W	1.181	0.323				
III	BL	3.132	0.584	1.78	10.750	0.00	S
	6 W	1.345	0.686				

The mean difference is significant at the 0.01 level (p<0.001)

Table 3. Comparison of mean BANA scores within the group before and after treatment

Group	Period	Mean	SD	Mean Change	Paired t-value	P-value	Sig
I	BL	1.400	0.843	1.00	3.87	0.004	S
	6 W	0.400	0.699				
II	BL	1.500	0.850	1.30	4.33	0.002	S
	6 W	0.200	0.422				
III	BL	1.500	0.707	1.30	6.09	0.000	S
	6 W	0.20	0.422				

The mean difference is significant at the 0.01 level (p<0.001)

Table 4. Comparison of mean Spirochete counts within the group before and after treatment

Group	Period	Mean	SD	Mean Change	Paired t-value	P-value	Sig
I	BL	36.30	5.59	23.70	12.979	0.00	S
	6 W	12.60	5.19				
II	BL	36.20	6.01	30.60	13.648*	0.00	S
	6 W	5.60	2.36				
III	BL	35.50	6.00	29.80	18.822*	0.00	S
	6 W	5.70	2.83				

* The mean difference is significant at the 0.01 level ($p<0.001$) A negative value indicates improvement

Discussion

Periodontal disease is a polymicrobial infection primarily caused by periodontal pathogens existing within the subgingival plaque[17]. The treatment of periodontal disease has primarily relied on mechanical therapy. With the continued evolution of specific plaque hypothesis, there has been increase in the adjunctive use of systemic antibiotics which have the ability to suppress or eradicate the pathogens. In addition, periodontal pathogens such as A actinomycetemcomitans and P.gingivalis that invade epithelium may not be accessible to standard mechanical debridement and require supplemental antibiotics and/or surgery for eradication[18]

Antimicrobial therapy should be used only when the clinical situation is serious enough to warrant this treatment and there is an appropriate diagnosis of an infection. The answer, then, as to whom to treat would be those patients who have multiple sites of inflammation associated with probing depths > 5 mm, and in whom a periodontal infection can be diagnosed.

Another type of patient to treat with antimicrobials would be the refractory patient. About 10 to 20% of patients are non-responsive to debridement procedures and access surgery and are considered as refractory to treatment (Hirshfeld and Wassermann, 1978; McFall,1982). Studies have shown that the adjunctive use of systemic antibiotics provide a better clinical outcome, particularly in terms of pocket depth reduction and attachment level gain than SRP alone[19,20] Hence, the present study was envisaged to determine and compare the efficacy of adjunctive use of systemic antibiotics (Azithromycin or Amoxicillin) with SRP and that of SRP alone and to evaluate the role of BANA as a tool in diagnosis of periodontal disease.

The present study has shown a reduction in the scores of plaque index (PI), bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment level (CAL) gain following periodontal therapy that was found to be statistically significant in all the groups. The reduction in spirochete count and BANA scores was found to correlate positively with the reduction in BOP, pocket depths and CAL gain. This is in accordance with the results of earlier studies, which have shown an improvement in clinical parameters subsequent to successful periodontal therapy.[21]

Overall, the results of the present study indicate that the judicial use of systemic antibiotics in the treatment of chronic periodontitis patients may provide an additional benefit in the clinical outcomes compared to SRP alone as they may prevent earlier recolonisation and reinfection of the sites due to sustained suppression of the periodontopathogens as evidenced by the significantly greater reduction in spirochete counts in subjects treated with systemic antibiotics as adjuncts to scaling and root planing.

Conclusion

The present study was designed to determine and compare the efficacy of systemic administration of Azithromycin or Amoxicillin as adjuncts to scaling and root planing with that of SRP alone. 30 subjects diagnosed with chronic periodontitis were selected and divided randomly into three groups. In Group I, subjects were treated with scaling and root planing alone and in Group II, subjects were treated with scaling and root planing along with systemic administration of Amoxicillin, while in Group III, subjects were treated with scaling and root planning along with systemic administration of Azithromycin. Periodontal parameters comprising of plaque index, bleeding index, probing pocket depth, clinical attachment level and microbiological parameters comprising of spirochete count and BANA test scores were assessed at baseline and six weeks after completion of periodontal therapy for subjects in all the three groups.

A base line comparison of all the parameters between the three groups did not show any significant difference. Following periodontal therapy, the periodontal health in all the subjects improved remarkably as evidenced by good plaque control, maintenance of gingival health, significant reduction in probing pocket depth and gain in clinical attachment level. This was also accompanied by significant reductions in spirochete count and BANA scores in all the three groups. However, subjects in Group II (SRP+AMOX) and in Group III (SRP+AZM) showed a greater reduction in spirochete count when compared to subjects in Group I (SRP alone) which was statistically significant.

BANA proved to be a reliable marker of disease activity as evidenced by the microbial count and clinical parameters. This study has shown that there is significantly greater reduction in spirochete count when antibiotics are used as adjuvants to scaling and root planning. Therefore, we can conclude that while mechanical debridement is an essential component of periodontal therapy, judicious use of antibiotics provides an added advantage. BANA hydrolysis is an effective and reliable tool for detecting chronic periodontitis and evaluating the outcome of therapy.

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