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A Comparative Evaluation of Efficacy of Amoxicillin and Azithromycin in the treatment of Chronic Periodontitis BANA Hydrolysis as a tool

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Abstract

***Aims and objectives*:** To compare the clinical and bacteriological changes occurring in chronic periodontitis after receiving azithromycin or amoxicillin as adjuncts to and scaling and root planning (SRP). alone at the base line and after 6 weeks completion of periodontal therapy.

Material and methods: Thirty subjects with chronic periodontitis with at least 8 sites with a probing depth of >4mm and attachment loss of ≥2 mm was selected and randomly divided in to three groups, ten in each group. Group I: SRP alone, Group II: SRP+ amoxicillin Group III: SRP+azithromycin. Clinical assessments and microbiological examination (dark field microscopic and enzyme assay BANA, (N-Benzoyl DL-Arginine 2-Naphthylamide) hydrolysis test) were carried, at the base line and 6 weeks after the completion of periodontal therapy.

***Results*:** A significant difference (P<0.001) was found in plaque index, BANA assay and in spirochete counts when assessed between the groups. The change in mean probing pocket depth values between the groups was not found to be statistically significant, and the border line significance was found in clinical attachment level gain.

***Conclusion*:** Systemic administration of antibiotics as adjuncts to scaling and root planning produces similar clinical outcome as that of scaling and root planning alone. A post-treatment comparison of all the parameters between the three groups failed to show any statistically significant difference except for the spirochete count. Subjects in Group II (SRP+AMOXICILLIN) and in Group III (SRP+AZITHROMYCIN) showed a greater reduction in spirochete count when compared to subjects in Group I (SRP alone).

Keywords: **P**eriodontitis, **S**caling and root planning, **A**djunctive therapy

INTRODUCTION

Periodontal diseases, now recognized as bacterial infections, are multi factorial infections, elicited by a complex of bacterial species, which interact with the host tissue cells, and release an array of cytokines,

chemokines and mediators leading to the destruction of periodontal supporting structures. affecting 5 to 30% of the adult population in the age group of 25 to 75+ years.[1] The knowledge about microbiological and immunological factors, responsible for the pathology of periodontal disease is increasing profoundly, leading to the development of certain indicators for identification of disease sites/persons, by using, phase contrast/ dark field microscopy, bacterial culture, immunoassays, nucleoid probes, enzyme assays and polymerase chain reaction assays.[2] Red complex species, which have unique properties possessing trypsin-like activity, detectable in sub gingival plaque samples, was found to have statistically significant association with the levels and proportions of spirochetes in plaque, with probing pocket depth, with clinical disease at site[3] and with immunological detection of PG, TF and TD in samples.[4] This trypsin-like enzyme is detectable only when there are sufficient numbers of colony forming units (CFU's) i.e. at least ten thousand CFU's. It hydrolysis the colorless synthetic substance BANA (N-Benzoyl DL-Arginine 2- Naphthylamide) and produce the chromogenic beta-naphthylamide which gives color with indicator dyes. Loesche WJ[5] proposed that the use of this BANA hydrolysis reaction to detect the presence of the periodontal pathogens could serve as a maker of the disease activity.

It is generally accepted that anti-infective therapy is the corner stone of periodontal therapy.[6] The local and systemically administered antimicrobial agents have shown to provide a better clinical outcome particularly in terms of pocket depth reduction and attachment level gain than scaling and root planing alone.[7] Hence, the present study was aimed to compare the clinical and bacteriogical changes occurring in chronic periodontitis subjects receiving either scaling and root planing alone or systemically administered azithromycin or amoxicillin as adjuncts to scaling and root planing.

MATERIALS AND METHODS

Subject population

A total number of 30 subjects were selected from the outpatient Dept. of periodontics, Government Dental College & Hospital. All patients had been informed about the study and written consent obtained prior to participation. Subjects who were in the age group of 25-55 years and having at least 20 natural teeth , who had not received any surgical / non-surgical periodontal therapy and any antibiotic therapy in past 6 months were included.

Experimental design and treatment

Subjects were diagnosed with Chronic generalized periodontitis when atleast 8 sites with a probing depth >4mm and attachment loss ≥2 mm were present.

The selected subjects were randomly assigned to one of the three Groups.

Group I (SRP alone): (n=10) subjects in this Group were treated only with scaling and root planing with out any systemic antibiotics.

***Group II (SPR+AMOX)*:** (n=10) subjects in this Group were treated with a combination of scaling and root planing along with systemic administration of Amoxicillin 250mg TID for 7 days.

Group III (SPRY+AZM) **:** (n=10) subjects in this Group were treated with a combination of scaling and root planing along with systemic administration of Azithromycin 500 mg, OD for 3 days.

Clinical assessments

The following clinical parameters were recorded for subjects in all the three groups. Plaque index[8] Bleeding Index[9] Probing pocket depth and Clinical attachment level.

Microbiological examination

Sample collection

The selected sites were isolated using sterile cotton rolls to avoid salivary contamination and were dried with sterile cotton pellets. Then clinically apparent supragingival plaque was removed from the sample site.

A sterile Gracey curette (Hu-friedy) was inserted gently to the apical pocket limit and then drawn coronally with sufficient force, to collect the most apically located subgingival plaque but not to plane the root Thereafter, the curette tip was vigorously agitated in a test tube containing 0.6 ml of Sorensen buffer solution at pH of 7.2, to dispense the entire plaque sample and dispersed for 20 s in a vertex mixer to get a homogenous plaque suspension.

Dark field microscopic examination

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A 10 µL of plaque suspension was placed on to a glass slide, covered by 22×30 cover slip to remove any entrapped air bubbles between slide and cover slip. This smear was examined under 10 x magnification of dark field microscope for evaluation of spirochetes within first one hour. The same smear was later stained with Fontana's dye for photographic record. The above examination was repeated for subjects in the entire three group after 6 weeks after treatment.

DISCUSSION

Periodontal disease is a polymicrobial infection primarily caused by periodontal pathogens existing within the subgingival plaque. In sub gingival plaque, diverse bacteria exist and extremely complex microbial flora is found.[10] The treatment of Periodontal disease has primarily relied on mechanical therapy i.e. root debridement performed either with or without surgical access to reduce overall plaque mass.[11] Studies have also 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.[12] Hence, the present study was envisaged to determine and compare the efficacy of adjunctive use of systemic antibiotics (Azithromycin or Amoxicillin) with that of SRP alone and to compare the efficacy of Azithromycin with that of Amoxycillin.

The present study has shown a reduction in the scores of plaque index (PI), bleeding on probing (BOP), probing pocket depth (PPD) and with attachment level (AL) gain following periodontal therapy that was found to be statistically significant. This is in accordance with the results of earlier studies, which have shown an improvement in clinical parameters subsequent to successful periodontal therapy.[13] The present study has shown a significant reduction in spirochete count and BANA scores following mechanical debridement of root surface with or without systemic use of antibiotics (AZM or AMOX). This is in accordance with the results of earlier studies, which have produced a similar outcome following periodontal therapy.[14]

The present study showed a positive correlation between the BANA scores and the spirochete count indicating that the reduction in BANA scores correlated with the reduction in spirochete count and this was found to be statistically significant. This is in accordance with the results of studies which have shown that a positive BANA test is indicative of elevated spirochete count and which correlates with the clinical signs of periodontal disease.[15] Several bacteriological studies such as by Loesche et al[16] and Slots J et al[17] have shown that the enzyme test could help the clinician to make an objective diagnosis of an anaerobic infection associated with these BANA positive species.[12]

The results of the present study showed a positive correlation between spirochete count, BANA scores and other periodontal parameters such as bleeding on probing (BOP), probing pocket depth (PD) reduction and clinical attachment level (CAL) gain. The reduction in spirochete count and BANA scores was found to correlate positively with the reduction in BOP, pocket depths and CAL gain. These results are in agreement with the results of earlier studies, which have shown a significant improvement in the clinical outcome following the reduction in microbial parameters.[18]

Though there was a significant improvement in periodontal parameters and reduction in spirochete count and BANA scores in all the three groups (SRP alone, SRP+ AZM and SRP+ AMOX), there was no statistically significant difference in the out comes when compared between the Groups. This finding strengthens the findings of the earlier studies, which have not shown any difference in the clinical outcomes of chronic periodontitis patients treated with either SRP alone or with SRP combined with systemic antibiotics.[19] Studies. have shown better clinical outcome when adult periodontitis patients were treated with systemic antibiotics as an adjunctive to SRP. [20]

In the present study , though the spirochete count decreased significantly in all the three groups (SRP alone, SRP+AMOX and SRP+AZM), it was found to be relatively more reduced in subjects treated with systemic antibiotics. It is plausible to hypothesize that there may be a chance for earlier recolonization and reinfection of the sites in the group treated with SRP alone. Studies have shown that the suppression in periopathogenic bacteria obtained when systemic antibiotics were given as adjuncts to scaling and root planning was more sustained than that obtained by scaling and root planing alone.[21] Overall, the results of the present study indicate that the routine use of systemic antibiotics in the treatment of chronic periodontitis patients may not provide any additional

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benefit in the clinical outcomes compared to SRP alone but may prevent earlier recolonisation and reinfection of the sites.

CONCLUSION

Data from the present study indicates that systemic administration of antibiotics as adjuncts to scaling and root planing produces similar clinical outcome as that of scaling and root planing alone. A post-treatment comparison of all the parameters between the three groups failed to show any statistically significant difference except for the spirochete count. 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). Prudent administration of antimicrobial agents following judicious pharmacological principles will preclude the abuse of chemotherapeutic agents and reduce the nature of developing drug resistance bacterial strains.

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Mean (± SD) Clinical and demographic features of subjects with data in three treatment groups at base line

Treatment groups

	SRP	Amoxicillin	Azithromycin
AGE	44.1	38.8	35
Males	6	6	3
Females	4	4	7
Plaque Index	1.63	1.66	1.19
Bleeding on Probing	65.63	65.39	69.86
Mean Pocket depth(mm)	5.02	5.03	5.10
Mean attachment level(mm)	3.02	3.04	3.13
Mean BANA score	1.4	1.5	1.5
Mean Spirochete %	36.30	36.20	35.50

Mean (± SD) Clinical and demographic features of subjects with data in three treatment groups after 6 weeks of treatment

	SRP	Amoxicillin	Azithromycin
AGE	44.1	38.8	35
Males	6	6	3
Females	4	4	7
Plaque Index	0.53	0.37	0.42
Bleeding on Probing	27.40	26.39	23.60
Mean Pocket depth(mm)	3.52	3.17	3.34
Mean attachment level(mm)	1.72	1.18	1.34
Mean BANA score	0.40	0.20	0.20
Mean Spirochete %	12.60	5.60	5.70

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Table I: Comparison of mean Plaque Index Scores within the group before (BL) and after treatment (6W)

GROUP	Period	Mean	Std.div	Mean Change	Paired t-value	P-value	Sig
I	BL	1.63	0.60	1.09	5.44*	0.00	S
	6 W	0.53	0.18				
II	BL	1.66	0.54	1.29	8.26*	0.00	S
	6 W	0.37	0.11				
III	BL	1.19	0.40	0.76	6.71*	0.00	S
	6 W	**0.42**	**0.19**				

*The Mean difference is significant at the0.01 level (p<0.001)

Table: IA Comparison of mean changes in Plaque Index scores between the groups before treatment by ANOVA

GROUP	SS	Df	Mean Square	F-value	P-value	Sig
Between Groups	1.42	2	0.71	2.56	0.095	NS
With in Groups	7.48	27	0.27			
Total	**8.91**	**29**				

Table: II Comparison of mean bleeding index scores within the group before and after treatment

GROUP	Period	Mean	Std.div	Mean Change	Paired t-value	P-value	Sig
I	BL	65.63	22.07	38.23	4.89*	0.00	S
	6 W	27.40	6.87				
II	BL	65.39	12.17	38.99	11.01*	0.00	S
	6 W	26.39	11.87				
III	BL	69.86	17.29	46.26	6.58*	0.00	S
	6 W	**23.60**	**13.68**				

* The Mean difference is significant at the0.01 level (p<0.001)

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Table: IIA: Comparison of mean changes in Bleeding index scores between the groups before treatment by ANOVA

GROUP	SS	Df	Mean Square	F-value	P-value	**Sig**
Between Groups	126.527	2	63.264	0.203	0.817	**NS**
With in Groups	8412.959	27	311.591			
Total	**8539.486**	**29**				

Table: IIB: Comparison of mean changes in Bleeding index scores between the groups after treatment ANCOVA (pair wise comparisons)

GROUP	Group compared with	Mean difference	P-value	**Sig**
I	II	1.02	0.837	**NS**
	III	3.51	0.483	**NS**
II	I	-1.02	0.837	**NS**
	III	2.48	0.619	**NS**
III	I	-3.51	0.483	**NS**
	II	**-2.48**	**0.619**	**NS**

A negative value indicates improvement.

Table: III: Comparison of mean Probing Pocket depth (mm) within the groups before and after treatment

GROUP	Period	Mean	Std.div	Mean Change	Paired t-value	P-value	**Sig**
I	BL	5.02	0.49	1.50	9.65*	0.00	**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*	0.00	**S**
	6 W	**3.34**	**0.80**				

* The Mean difference is significant at the0.01 level (p<0.001)

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Table: III A: Comparison of mean changes Probing Pocket depth (mm) between the groups before treatment by ANOVA

GROUP	SS	Df	Mean Square	F-value	P-value	**Sig**
Between groups	0.038	2	0.019	0.069	0.934	**NS**
With in Groups	7.547	27	0.280			
Total	**7.585**	**29**				

Table: III B: Comparison of mean changes in Probing Pocket depth (mm) between the groups after treatment ANCOVA (pair wise comparisons)

GROUP	Group compared with	Mean difference	P-value	**Sig**
I	II	0.349	0.272	**NS**
	III	0.181	0.564	**NS**
II	I	-0.349	0.272	**NS**
	III	-0.167	0.595	**NS**
III	I	-0.181	0.564	**NS**
	II	**1.674**	**0.595**	**NS**

A negative value indicates improvement.

Table: IV: Comparison of mean CAL (mm) within the group before and after treatment

GROUP	Period	Mean	Std.div	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 the0.01 level (p<0.001)

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Table: IVA: Comparison of mean changes in CAL (mm) between the groups before treatment by ANOVA

GROUP	SS	Df	Mean Square	F-value	P-value	**Sig**
Between Groups	0.063	2	0.032	0.089	0.915	**NS**
With in Groups	9.650	27	0.357			
Total	**9.713**	**29**				

Table: IVB: Comparison of mean changes in CAL(mm) between the groups after treatment ANCOVA(pair wise comparisons)

GROUP	Group compared with	Mean difference	P-value	**Sig**
I	II	0.542	0.049*	**S**
	III	0.379	0.162	**NS**
II	I	-0.542	0.049*	**S**
	III	-0.163	0.162	**NS**
III	I	-0.379	0.049	**NS**
	II	**0.163**	**0.540**	**NS**

A negative value indicates improvement.

Table: V: Comparison of mean BANA scores within the group before and after treatment

GROUP	Period	Mean	Std.div	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 the0.01 level (p<0.001)

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Table: VA: Comparison of mean changes in BANA scores between the groups before treatment by ANOVA

GROUP	SS	Df	Mean Square	F-value	P-value	**Sig**
Between Groups	0.067	2	0.033	0.052	0.950	**NS**
With in Groups	17.40	27	0.644			
Total	**17.467**	**29**				

Table: VB: Comparison of mean changes in BANA scores between the groups after treatment ANCOVA(pair wise comparisons)

GROUP	Group compared with	Mean difference	P-value	**Sig**
I	II	0.20	0.407	**NS**
	III	0.20	0.407	**NS**
II	I	-0.20	0.407	**NS**
	III	0.00	1.00	**NS**
III	I	-0.20	0.407	**NS**
	II	**0.00**	**1.00**	**NS**

A negative value indicates improvement.

Table: VI Comparison of mean Spirochete counts within the groups before and after treatment

GROUP	Period	Mean	Std.div	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 the0.01 level (p<0.001)

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Table: VIA: Comparison of mean changes in Spirochete counts between the groups before treatment by ANOVA

GROUP	SS	Df	Mean Square	F-value	P-value	**Sig**
Between Groups	322.06	2	161.03	11.91	0.00*	**S**
With in Groups	364.90	27	13.51			
Total	**686.967**	**29**				

Table: VI B: Comparison of mean changes in Spirochete counts between the groups after treatment by ANCOVA(pair wise comparisons)

GROUP	Group compared with	Mean difference	P-value	**Sig**
I	II	7.00*	0.00	**S**
	III	6.90*	0.00	**S**
II	I	-7.00*	0.00	**S**
	III	-0.10	0.952	**NS**
III	I	-6.90*	0.00	**S**
	II	**0.10**	**0.952**	**NS**

A negative value indicates improvement.

* The Mean difference is significant at the0.01 level (p<0.001)

Table VII : Pearson's Correlation Co-efficient "r'' matrix of the overall changes between all the variables

Variable	**PI Change**	**Bleding**	**PD Change**	**CAL Change**	**BANA Change**	**% of Spirochete change**
BANA Change	0.176	0.288	0.246	0.177	1	**0.654****
% of Spirochete change	**-0.240**	**0.178**	**0.337***	**0.342***	**0.654****	**1**

** Co-relation is significant at 0.001 level (I-tailed)

* Co-relation is significant at 0.005 level (I-tailed)

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