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Effects Of Scaling And Root Planing And 0.2 % Chlorhexidine Rinse On Clinical And Microbiological Parameters In Generalised Chronic Periodontitis– A Clinico - Microbiological Study.

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ABSTRACT

Background: 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-planning (SRP) are well documented .Antimicrobial agents act as an adjunct to periodontal therapy. One of the most frequently used antimicrobial agents is chlorhexidine gluconate (CHX), it is a broad spectrum antiseptic with a pronounced antimicrobial effect 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, an enzyme capable of hydrolyzing synthetic trypsin substrate, BANA. **Methods**: This study included 30 individuals who were randomly distributed in two groups test (SRP + CHX) and control (SRP) **Results**: The results of the study stated that the treatment with SRP and CHX improved clinical and microbiological parameters compared to the SRP alone as a monotherapy. **Conclusion**: CHX rinsing and repeated professional plaque removal could have equivalent therapeutic benefits, the use of CHX offers the great advantage of not requiring the patient's presence in the dental office.

Keywords: Scaling and root-planning (SRP), chlorhexidine gluconate (CHX), (N-benzoyl-d L-arginine-2-napthylamide) BANA

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] 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-planning (SRP) are well documented.[2,4] 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 Haffajee et al.[5] reported that SRP alone has limited effect on some pathogenic species.[6] Microbiological techniques demonstrated that the combination of SRP and repeated professional plaque removal could have a beneficial effect on the subgingival microbiota.[7,8] This has led to use of antimicrobial agents as an adjunct to periodontal therapy. One of the most frequently used antimicrobial agents is chlorhexidine gluconate (CHX), it is a broad spectrum antiseptic with a pronounced antimicrobial effect on both gram negative and gram positive bacteria as well as on some yeast and lipophillic viruses and its prolonged substantivity is still recognized as the gold standard for chemical plaque control.[9] 0.2% chlorhexidine solution was the first clinically effective mouth rinse that inhibited supragingival plaque formation.[10]

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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, invivo an enzyme capable of hydrolyzing synthetic trypsin substrate, BANA (N-Benzoyl D-L Arginine -2 Naphalamide) .BANA a colourless 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]

MATERIALS AND METHODS

The current study was performed on 30 subjects randomly selected comprising of visiting outpatient department of Periodontology, Govt. Dental College and Hospital Srinagar. All these subjects were considered for the clinical study after meeting inclusion and exclusion criteria. The criteria for inclusion in the study were that the age of subjects had to be between 25-50 years. All those who were diagnosed as suffering from generalized chronic periodontitis determined on clinical and radiographic examination with minimum of 4 teeth with one site with pocket depth ≥5mm & ≤7mm involvement. Cooperative patients who are able to attend the hospital at frequent intervals and giving a positive consent. However the exclusion criteria included that any 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 7 days or longer in previous 3 months were excluded.

Before the selected subjects were taken up for the study, study protocol and benefits were explained to them. All those who volunteered and signed a written consent were included in the study. After the random selection of 30 subjects for the study based on the inclusion and exclusion criteria, the periodontal examination was done. Subjects were randomly assigned into two groups, 15 Subjects in Control Group (Group A) and 15 Subjects in Treatment Group (Group B). The groups were named as, The Control group- Group A (SRP + PLACEBO) and treatment group- Group B (SRP + 0.2% CHX) At baseline the recording were made for four-Non adjacent periodontal pockets in posterior segment of mouth measuring depth ≥5mm & ≤7mm were assessed. Following periodontal parameters will be recorded in both groups (Group I and Group II) at BASELINE: Plaque index (Sillness and Loe, 1964).[13] Sulcus bleeding index (Muhlemann HR and son,1971).[14] Probing Pocket depth. (with Williams Graduated Periodontal Probe),[15] Relative Attachment level i.e. distance between base of sulcus or pocket and a fixed reference point (horizontal notch) on the acrylic stent and microbiological parameters were assessed using BANA Test (N-benzoyl-d L-arginine-2-napthylamide).[12,15,16]

Subgingival plaque sample was collected at baseline from the selected site with the pocket depth of ≥5mm& ≤7mm with Gracey curettes then was placed on BANA impregnated strips at the lower half of the strip . [Figure 1] The upper reagent matrix contains a chromogenic diazo reagent (fast black K) was activated by moistening it with distilled water. The upper reagent matrix, which reacts from BANA by bacterial enzyme reacts with fast black K forming a permanent blue color when incubated at 55°C for 15 mins. The blue color of a positive or weak positive reaction appears in the upper matrix and is permanent.[12] The BANA strips were subsequently examined for the presence (positive) or absence (negative) of blue colour. No colour change was marked for negative, light faint blue was marked for weak positive and evident blue marked for positive test result.

Second recordings for recording of periodontal parameters were made at day 21. Following periodontal parameters were recorded in both groups (Group A and Group B) at day 21. Plaque index (Sillness and Loe, 1964).[13] Sulcus bleeding index (Muhlemann H.R and son ,1971).[14] Reinforce oral hygiene instructions. A gap of 3days for CHX rinses. At day 42 the third recording in both groups (Group A and Group B) was made for the similar periodontal parameters as were made on the 21st day of study. Biological parameters were recorded in similar manner by utilizing BANA (N-benzoyl-d L-arginine-2-napthylamide) method.[12,16] The intervention was controlled by a single examiner who conducted the study. The Control group(Group A – SRP + PLACEBO) received oral hygiene instructions and full mouth scaling using ultra-sonic scaler (magnetostrictive) followed by root planing using Gracey curettes performed under local anaesthesia if required. The subjects in the group were put on normal saline rinses after completion of periodontal therapy till 42nd day. Treatment group(Group B - SRP + 0.2% CHX) Received oral hygiene instructions and full mouth scaling using ultrasonic scaler (magnetostrictive) followed by root planing using Gracey curettes performed under local anaesthesia

if required. The subjects in this group were put on 0.2% chlorhexidine rinses 15 ml twice a day after completion of periodontal therapy till 42nd day with a gap of 3 days on 21st day to reduce the side effects of CHX.

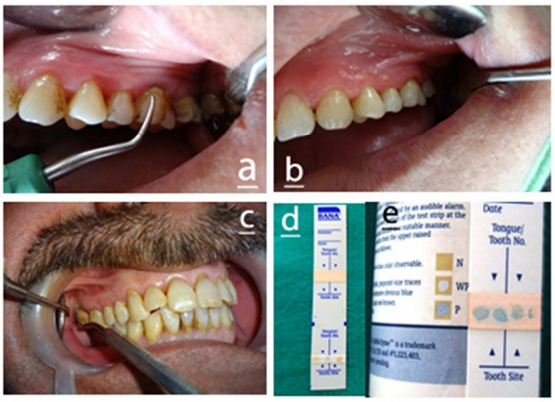

Figure 1: a) Scaling done using Magnetostrictive Ultrasonic Scaler, b)After Scaling, c) Collecting of subgingival plaque sample, d) Plaque sample placed over the BANA strip & strip folded, e) Reagent strip results after incubation matched with the indicator on the bottle.

Stringent oral hygiene instructions were given advocating brushing once daily with same dentifrice in both control and test group using Bass method. Use of no adjunctive interdental aids in both test and control. Use of mouthwash half an hour after breakfast and tooth brushing and at night before going to sleep.

The data was collected at the given intervals after the baseline data collection. Data was entered in Microsoft Excel work sheets and the statistical analysis was done by using Statistical Software SPSS (Version 20.0). Data was analysed by applying descriptive statistics viz., means, standard deviations and percentages. 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

Out of the 30 subjects selected for the present study, the mean recordings for plaque index are recorded in table 1. Mean plaque index of control group (Group A- SRP +PLACEBO) at baseline was 1.8805 at day 21 it was 1.4297, at day 42 was 1.2195. Mean plaque index of test group (Group B- SRP +CHX) at baseline was 1.9164 at 21 day was 1.2756 and 42 day was 0.987. Reduction in mean plaque index scores in the control group (Group A) at different time intervals is seen in [Table 1]. The difference in the mean plaque index scores between baselines to 21st day was 0.4508 with p value 0.006 which is statistically significant. From baseline to 42nd days the difference in mean plaque scores are 0.6610 with p value <0.001 highly significant. [Table 1] presents the reduction in mean plaque index scores in the control group (Group B) at different time intervals. The difference in the mean plaque index scores in this group between baseline to 21st day was 0.6408 with p value <0.001 which is highly significant. From baseline to 42nd day the difference in mean plaque scores are 0.9293 with p value <0.001 highly significant. On comparison between the two groups at the baseline the difference showed no statistical difference. 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 decrease at day 21 (p 0.001) and statistically highly significant decrease at day 42 (p < 0.001).

Table 1: Comparison of mean Plaque Index reduction scores between two groups

Plaque Index	Group A		Group B		Difference between groups	P-value
	Mean	SD	Mean	SD		
Baseline	1.8805	0.0745	1.9164	0.1687	0.0359	0.490#
21 Days	1.4297	0.0808	1.2756	0.1013	0.1541	0.001*
42 Days	1.2195	0.0883	0.9871	0.1242	0.2324	<0.001*

Bleeding on probing was assessed by Sulcus Bleeding Index. Mean sulcus bleeding index of control group (Group A- SRP +PLACEBO) at baseline was 1.8805, at day 21 was 1.1004,at day 42 was 0.9479.Mean plaque index of test group (Group B- SRP +CHX) at baseline was 1.9168 at 21 day was 0.9839 and 42nd day was 0.7900. Reduction in mean sulcus bleeding index scores in the control group (Group A) at different time intervals is presented in Table 2. The difference in the mean sulcus bleeding index scores between baseline to 21 day was 0.7704 with p value 0.003 which is statistically significant. From baseline to 42 days the difference in mean sulcus bleeding index scores are 0.9229 with p value <0.001 highly significant. Reduction in mean sulcus bleeding index scores in the test group (Group B) at different time intervals is shown in table 2. The difference in the mean sulcus bleeding index scores between baseline to 21day was 0.9329 with p value <0.001 which is statistically highly significant. From baseline to 42 days the difference in mean sulcus bleeding index scores are 1.1268 with p value <0.001 highly significant. 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 21 (p 0.004) and statistically highly significant decrease at day 42 (p < 0.001).

Table 2: Comparison of mean Sulcus Bleeding Index reduction scores between two groups

Sulcus Bleeding Index	Group A		Group B		Difference between groups	P-value
	Mean	SD	Mean	SD		
Baseline	1.8708	0.0650	1.9168	0.0840	0.0460	0.131#
21 Days	1.1004	0.0734	0.9839	0.1076	0.1165	0.004*
42 Days	0.9479	0.1216	0.7900	0.0917	0.1580	0.001*

*Statistically Significant Difference (P-value by Independent t-test)
#Statistically Non-significant Difference (P-value by Independent t-test)

Mean probing pocket depth in control group (Group A- SRP +PLACEBO) at baseline was 6.82, at day 42 was 4.31[Table 3]. Mean probing pocket depth in test group (Group B- SRP +CHX) at baseline was 6.89, at 42 day was 3.90 In control group (group A) the reduction in mean probing pocket depth from the baseline to 42 days was 2.51mm with a p value <0.001 which is statistically highly significant. In test group (group B) the reduction in mean probing pocket depth from the baseline to 42 days was 2.99mm with a p value <0.001 which is statistically highly significant. On comparison between the two groups at the baseline the difference was statistically not significant. Whereas at day 42 nd 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).

Table 3: Comparison of Mean Probing Pocket Depth reduction scores between two groups

Probing Pocket Depth	Group A		Group B		Difference between groups	P-value
	Mean	SD	Mean	SD		
Baseline	6.82	0.153	6.89	0.062	0.07	0.175#
42 Days	4.31	0.311	3.90	0.514	0.41	0.013*

*Statistically Significant Difference (P-value by Independent t-test) #Statistically Non-significant Difference (P-value by Independent t-test)

[Table 4] shows the relative attachment levels at different time intervals in the groups. Mean relative attachment level in control group (Group A- SRP +PLACEBO) at baseline was 9.82, at day 42 was 7.26. Mean relative attachment level in test group (Group B- SRP +CHX) at baseline was 9.89, at 42 day was 6.93. Reduction in mean relative attachment level scores in the control group (Group A) at different time intervals. In control group (group A) the reduction in mean relative attachment levels or the mean gain of attachment from the baseline to 42 days was 2.56mm with a p value of <0.001 which is statistically highly significant. Reduction in mean relative attachment level scores in the test group (Group B) at different time intervals. In test group (group B) the reduction in mean relative attachment levels or the mean gain of attachment from the baseline to 42 days was 3.32 mm with a p value of <0.001 which is statistically highly significant. On comparison between the two groups at the baseline. The difference was statistically not significant. The test grp (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).

Table 4: Comparison of changes in Mean Relative Attachment Levels between two groups

Relative Attachment Level	Group A		Group B		Difference between groups	P-value
	Mean	SD	Mean	SD		
Baseline	9.82	0.151	9.89	0.062	0.07	0.146#
42 Days	7.26	0.343	6.93	0.332	0.33	0.012*

*Statistically Significant Difference (P-value by Independent t-test)
#Statistically Non-significant Difference (P-value by Independent t-test

Evaluation of changes in the subgingival microbiota was assessed by BANA test [Table 5]. In group A at baseline %age of sites with score 0 (negative result) is 0, at 42nd day the %age is increased to 65%. The %age of sites with score 2 (positive result) is 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 [Table 5] at baseline % of sites with score 0(negative result) is 0 , at 42 day the % age is increased to 73.3%.The % age of sites with score 2 (positive result) is 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 [Table 5] 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.

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 the control group. Both groups had a reduced frequency of BANA-positive sites and increased frequency of BANA-negative sites. These results were more marked and better sustained over time in subjects who rinsed with CHX.

Table 5: Comparison between two groups based on BANA test score

	BANA	Group A (n=54 sites)		Group B (n=54 sites)		P-value
		No.	%age	No.	%age	
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)

DISCUSSION

The current prospective parallel study was performed on 30 Subjects randomly selected, visiting outpatient department of Periodontology, Govt. Dental College and Hospital Srinagar. The study was completed in 42 days from baseline.

All the subjects completed the study and there was no dropout of any study subject.

It was seen that subjects from the combined therapy group (Group B) exhibited the greater reduction in the plaque scores at 42 day ($p<0.001$). These results were found to be consistent with the previous studies that confirm the antiplaque efficiency of CHX rinses.[17,18] The difference in reduction in plaque index could be due to demonstrated action of 0.2% CHX in inhibiting supragingival plaque formation and the development of gingival inflammation.[19] The superior antiplaque activity of chlorhexidine has been attributed to its property of "substantivity", resulting in the "Bacteriostatic mileu" in the oral cavity for upto 8-12 hours.[20,21]

Furthermore, in the present study it was seen that subjects from the combined therapy (Group B) group exhibited the greater reduction in the sulcus bleeding index scores at 42nd day ($p<0.001$). These results were found to be consistent with the studies of that confirm the antinflammatory effect of CHX rinses.[17,18] The greater difference in reduction in sulcus bleeding index scores could be also attributed to decrease in gingival inflammation

Significant difference (p value <0.05) in the mean reduction in probing pocket depth between Group A, Group B at baseline and 42nd were recorded. These results were found to be consistent with previous studies.[22] Reduction could be because of maintainance of strict supragingival plaque control in a previously cleaned site effectively retards the recolonization of subgingival plaque.[23] Thus the effect of CHX in altering subgingival microbiota by preventing the recolonization of putative periopathogens into the pocket. Also the property of "substantivity" of CHX,[20] thereby creating more healthy periodontal environment for reduction of pocket depth.

The mean gain in attachment level in both groups between baseline and 42nd day was highly significant (p value <0.01). These results were consistent with previous studies Attachment gain subsequent to subgingival scaling and improvement in oral hygiene appears to be due to the migration of dentogingival junction to or close to the apical level of root instrumentation during healing due to the reduction in the tissue inflammation following the removal of plaque and calculus,[2] and oral hygiene instructions that were given to the subjects. These results were found to be consistent with previous studies.[22]

Although the reduction in group B was significantly more this could be due to maintenance of strict supragingival plaque control in a previously cleaned site which effectively retards the recolonization of subgingival plaque.[23] Thus the effect of CHX in altering subgingival microbiota and its property of "substantivity "helps healthy periodontal environment for attachment gain.

The BANA test has been successfully used to evaluate changes in the subgingival microbiota.[24,25] This technique detects the presence of arginine hydrolase, an enzyme produced by P. gingivalis, T. denticola and T. forsythia,.[25,26] In the present study both groups showed a significant post therapy reduction in the frequency of the anaerobic microorganisms at 42 day when compared to the baseline ($p<0.001$). The results were consistent with previous studies.[27] This could be due to effect of SRP on subgingival microflora which causes significant reduction in percentage of spirochetes, total motile organisms, fusobacterium sp.[28] However, an additional microbiological benefit was observed, that these

subjects showed a greater reduction in the percentage of BANA-positive sites, a greater increase in the % of BANA-negative sites 42 days in comparison with the control group. This was in accordance with the study.[22] This could be due to the antimicrobial effect of CHX. The better clinical and microbiological effects observed with the combination of SRP and CHX in this study might be attributed to a beneficial effect of CHX rinsing on subgingival microbial recolonization. Maintaining low levels of supragingival plaque and disturbing periodontal pathogen reservoirs that are not reached by SRP, such as tonsils, tongue, saliva and oral mucous membranes,[29,30] could have a positive influence on recolonization of the recently scaled pockets.

It can be concluded from the results of the present study that the treatment with SRP and CHX improved clinical and microbiological parameters compared to the SRP alone as a monotherapy. These results could be attributed to the favorable environment created by anti-inflammatory antimicrobial effect of CHX which prevented the re-colonization of putative pathogenic bacteria by creating a "Bacteriostatic mileu". Hence, to conclude CHX rinsing and repeated professional plaque removal could have equivalent therapeutic benefits, the use of CHX offers the great advantage of not requiring the patient's presence in the dental office. The better clinical and microbiological effects observed with the combination of SRP and CHX in this study might be attributed to a beneficial effect of CHX rinsing to decrease microbial recolonization.

CONCLUSION

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