Evaluation of the Efficacy of Chlorhexidine Chip as an Adjunct to SRP in the Treatment of Chronic Periodontitis: A Clinical and Microbiological Study

Dr. Ranjana Das[1], Dr. Himanshu Khashu[2], Dr. Richa Agrawal[3] ,Dr. Ajay Chouksey[4] , Dr. Manoj Tiwari[5] , Dr. Sandeep Nagar[6]

[1]Post graduate student, Department of Periodontology, Mansarovar Dental College, Bhopal, Madhya Pradesh, India
[2]Professor and Head, Department of Periodontology, Mansarovar Dental College, Bhopal, Madhya Pradesh, India
[3]Professor, Department of Periodontology, Mansarovar Dental College, Bhopal, Madhya Pradesh, India
[4]Reader, Department of Periodontology, Mansarovar Dental College, Bhopal, Madhya Pradesh, India
[5]Reader, Department of Periodontology, Mansarovar Dental College, Bhopal, Madhya Pradesh, India
[6]Post graduate student, Department of Periodontology, Mansarovar Dental College, Bhopal, Madhya Pradesh, India

Abstract

Aim: Chlorhexidine (CHX) is commonly used by dental practitioners and the public, due to its antimicrobial effects. The aim of this study was to Evaluate the efficacy of chlorhexidine chip as an adjunct to SRP in the treatment of chronic periodontitis.

Material and method: A study was conducted by the Department of Periodontics, Mansarovar Dental College and Hospital, Bhopal to evaluate the clinical and specific microbiological changes associated with chlorhexidine chip (DSI Sponge Perio, DSI Ltd, Ashdod, Israel) in chronic periodontitis patients. Twenty patients with chronic periodontitis and periodontal pockets $\geq$ 4 mm. were randomly selected from the outpatient department. Patients were divided into two groups, each group contained ten patients. A 3 months simple randomized, clinical study was conducted comparing the effect of SRP with and without chlorhexidine chip in chronic periodontitis patient and the outcomes present in the probing depth (PD), gingival_index (GI) and clinical attachment level (CAL) , AA and P.gingivalis were evaluated. Both clinical and microbiological recordings were carried out at baseline, 1 and 3 months post-treatment.

Result: Periodontal parameters showed significant difference for Gingival index, Periodontal index, probing pocket depth, clinical attachment loss and mSBI at 3 months between Group A and Group B. P. gingivalis showed significant mean difference of 18.48000 at 3 months between groups at p=0.003. A. actinomycetemcomitans was found to be higher in Group 1 with a mean microbial count of 115.4000 X 10^3 while Group 2 had a mean of 81.2000 X 10^3 , which was significant at p=0.000.

Conclusion: Local drug delivery using chlorhexidine chip enhances the benefit of SRP in the treatment of chronic periodontitis.

Keywords: Chlorhexidine chip, Local drug delivery, Microbiological study, P. Gingivalis, A. Actinomycetemcomitans, simple randomized clinical Trial

Introduction

Periodontitis is an infectious disease characterized by inflammatory changes in the surrounding tissues leading to periodontal attachment loss and alveolar bone destruction.[1]

Periodontal therapy is directed at disease prevention, slowing or arresting disease progression, regenerating lost periodontium and maintaining achieved therapeutic objectives.[2] Successful periodontal therapy is dependent on anti-infective procedures aimed at eliminating pathogenic organisms found in the dental plaque associated with the tooth surface and with other niches in the oral cavity.[3]

A number of treatment modalities are available for treating periodontitis and have shown consistent results. Sub-gingival debridement in the form of scaling and root planing remains an essential part of successful periodontal therapy. Non-surgical mechanical treatment is the corner stone of periodontal therapy and the first recommended approach for the control of periodontal infection. Although nonsurgical periodontal therapy has evolved over the years, it is still considered to be the gold standard to which other treatment methods are compared.[4]Overall, SRP appeared to have a moderate effect on composition of sub-gingival microbiota. SRP showed significant decrease in the levels of P.gingivalis,

A.actinomycetemcomitans , B.forsythus and T.denticola post-therapy.[5]

Several investigators have hypothesized that active chlorhexidine must both reach the site of action (i.e. the base of the pocket.) and remain active in the pocket for a time to inhibit the growth of pathogenic bacteria.[6] Chlorhexidine per se is a potent antimicrobial drug and inhibits the growth of periodontal bacteria in vitro at concentrations that may be achieved by controlled delivery.[7] Number of studies exists in the literature comparing SRP with local delivery of chlorhexidine in treating periodontitis cases. [8,9] Hence the present study is planned to compare the treatment outcomes of two nonsurgical treatment modalities i.e. Scaling and root planing, and local drug delivery with chlorhexidine as an adjunct to SRP in treating chronic periodontitis.

Material and methods

Total of 20 patients with 3 sites each, hence a total of 60 sites will be enrolled in this study who will be attending the department of periodontics, Mansarovar Dental College, Bhopal. The nature and purpose of the study will be explained to the patients and an informed consent will be obtained. At baseline all the patients are screened for COVID-19. A detailed case history will be recorded in a specially prepared proforma which will include information regarding the patient's overall medical health and oral health. After baseline examination, a simple randomization by using chit method will be done to assign patients in a split mouth design to one of the following 3 treatment modalities:

Group I: Scaling and root planing only (SRP group).

Group II: Chlorhexidine chip as an adjunct to Scaling and root planing (Chlorhexidine chip group).

In all the patients full mouth nonsurgical periodontal therapy will be done comprising of a single session of scaling and root planning with ultrasonic instruments (EMS)and Gracey curettes (Hu-Friedy, USA) along with oral hygiene instructions under local anaesthesia using for the teeth in group I no additional treatment will be provided other than conventional scaling and root planing . For teeth in Group II as an adjunctive to the conventional mechanical treatment, local drug delivery of chlorhexidine chip (PerioChip, Dexcel Pharma Technologies Ltd.) will be done according to the manufacturer's guidelines. The test site will be isolated and dried with compressed air for the placement of chlorhexidine chip, as wet chip becomes soft and more difficult to insert. It will be carried using tissue holding forceps, with round side of the chip facing away from forceps. Subgingival administration of chlorhexidine chip will be accomplished by inserting the round end of the chip directly into the base of the pocket. Chip will be pressed apically so that it rests sub gingivally at the base of the pocket. Patients will be advised not to use dental floss for 7 days to avoid displacement of the CHX chip and to avoid the use of chemotherapeutic mouth rinses during the study period. Clinical parameters will be recorded at Baseline, 1 and 3 months and oral hygiene instructions will also be reinforced in every visit.

Inclusion criteria were patients between the age group of 18-55 years with chronic generalized periodontitis with at least 3 teeth having probing pocket depth ≥ 5mm. Also patients should be in good general health with no history of systemic disorders and no periodontal therapy received during the past one year. And had not taken antibiotics during the past 6 months and who were willing and able to return for multiple follow-up visits.Exclusion criteria were patients under the age of 18, pregnant and lactating women, patients history of smoking/tobacco or alcohol consumption in past five years, patients allergic to CHX. Patients that received periodontal treatment in less than 3 months of the preliminary consultation and also patients on any chemo-therapeutic mouth rinses and oral irrigation during the past 6 months, who have received any surgical therapy 6 months prior to the start of the study are excluded.

A three months simple randomized, clinical study was conducted comparing the effect of SRP with and without chlorhexidine chip in chronic periodontitis patient and minimum follow up was at least 1 months of follow-up and the outcomes present in the probing depth (PD), gingival index (GI) and clinical attachment level (CAL) after scaling and root planning (SRP).

The nature and design of the clinical study was explained and informed consent was obtained from all the participants. The clinical parameters recorded in the proforma included: Loe and Silness gingival index (GI), Probing pocket depth (PPD), Clinical attachment levels (CAL) and Modified Sulcular Bleeding Index (mSBI). One molar site with pocket depth of ≥5mm was selected in each patient for the study.

On the first appointment, all patients received routine oral hygiene instructions & one-stage full-mouth

scaling & root debridement employing both hand instruments (Hu- Friedy, USA) & a piezoelectric ultrasonic handpiece under local anaesthesia of 2% lidocaine with 1:80000 adrenaline (XICAINE 2% A; ICPA HEALTH PRODUCTS LTD, Ankleshwar).

On second appointment i.e. after 24 hours complete SRP was performed for both Groups and subgingival placement of chlorhexidine chip was done after proper isolation of the area in Groups 2 and again all patients were given oral hygiene instructions.. Both clinical and microbiological recordings were carried out at baseline, 1 and 3 months post-treatment. All recordings were subjected for statistical analysis by using Pearson Chi-square test and Analysis of variance (ANOVA) test.

Microbiological Assessment:

Microbiological culture examination of anaerobic isolates. Sub-gingival GCF samples were taken from tooth with a pocket of ≥ 5mm with a sterile paper points at baseline and 3 months. Samples were obtained from the deepest periodontal pocket in each quadrant of the dentition by using sterile paper points. The samples were suspended in Eppendorf tube pooled in 1.5 ml. Reduced Transport Fluid i.e., sterile saline solution (0.85%) and processed for anaerobic cultivation within 4 h after sampling. The microbial analysis was carried out in the Center for Microbiology and Bio-Technology Research and Training Institute, Bhopal, (M.P.)

Identification of anaerobic isolates Microorganisms

For anaerobic culture 100 µl of appropriate 10-fold dilutions were plated on blood agar plates that were supplemented with horse blood (5% v/v), hemin (5 mg/l^{-1}) and menadione (1 mg/l^{-1}) and incubated in 80% N_2, 10% H_2 and 10% CO_2, at 37℃ for 7 up to 14 days. P.gingivalis were identified on the basis of colony morphology, black pigment, anaerobic growth, the inability to ferment glucose, indole production as well as the production of a set of metabolic enzymes. A. actinomycetemcomitans was grown on trypticase soy-serum-bacitracin- vancomycin (TSBV) plates and incubated at 37℃ in air + 5% CO_2 for 3 days. The identification of A. actinomycetemcomitans was based on its characteristic colony morphology and a positive catalase reaction with 3% hydrogen peroxide. The total number of colony forming units (CFU) per sample was determined.

Determination of the number of CFU per milliliter of the bacterial suspensions was made by growing the bacteria 2–3 days in Brain Heart Infusion (BHI) supplemented with 5 mg/l^{-1} hemin & 5 mg/l^{-1} menadione, and plating serial dilutions as described above. The strains used in this study were: P. gingivalis (W83) and A. actinomycetemcomitans (NCTC 9710).

Results:

Table 1: Age distribution between the groups

Groups	N	Mean	Std. Deviation
Group 1	10	35.0000	8.12404 (NS)
Group 2	10	33.7000	6.68414 (NS)

*=Significant; NS= Not significant

Table 2: Periodontal parameters in Group 1 at various intervals

Interval	Mean	Std. Deviation	Std. Error	ANOVA statistic	P value
Gingival Index					
Baseline	2.6000	.51640	.16330	9.661	.001*
1 month	1.7000	.48305	.15275		
3 months	1.4000	.84327	.26667		
Periodontal Index					

Baseline	2.2000	.63246	.20000	5.353	.011*
1 month	1.2000	.91894	.29059		
3 months	1.3000	.67495	.21344		
Probing pocket depth					
Baseline	5.9000	.99443	.31447	6.877	.004*
1 month	5.0000	.94281	.29814		
3 months	4.1000	1.28668	.40689		
Clinical Attachment level					
Baseline	5.4000	.69921	.22111	5.190	.012*
1 month	4.9000	.99443	.31447		
3 months	4.2000	.78881	.24944		
mSBI					
Baseline	2.5000	.52705	.16667	12.699	.000*
1 month	1.6000	.51640	.16330		
3 months	1.4000	.51640	.16330		

*=Significant; NS= Not significant

Group 1 subjects showed significant decrease in mean scores in all periodontal parameters as seen in Table 1. GI reduced to 1.4000 $\pm$ 0.84327 from 2.6000 $\pm$ 0.51640, PI to 1.3000 $\pm$ 0.67495 from 2.2000 $\pm$ 0.63246, PPD to 4.1000 $\pm$ 1.28668 from 5.900 $\pm$ 0.99443, CAL to 4.2000 $\pm$ 0.78881 and mSBI to 1.4000 $\pm$ 0.51640 from 2.5000 $\pm$ 0.52705.

Table 3: Periodontal parameters in Group 2(SRP +CHX CHIP) at various intervals

Interval	Mean	Std. Deviation	Std. Error	ANOVA statistic	P value
Gingival Index					
Baseline	2.5000	.52705	.16667	26.591	.000*
1 month	1.0000	.81650	.25820		
3 months	.5000	.52705	.16667		
Periodontal Index					
Baseline	2.4000	.69921	.22111	14.447	.000*
1 month	1.4000	.96609	.30551		
3 months	.6000	.51640	.16330		
Probing pocket depth					
Baseline	5.9000	.99443	.31447	32.920	.000*
1 month	4.1000	.73786	.23333		
3 months	2.9000	.73786	.23333		
Clinical Attachment level					
Baseline	5.5000	.52705	.16667	49.867	.000*
1 month	3.6000	.69921	.22111		
3 months	2.9000	.56765	.17951		
mSBI					
Baseline	2.6000	.51640	.16330	22.680	.000*
1 month	2.0000	.81650	.25820		
3 months	.8000	.42164	.13333		

*=Significant; NS= Not significant

Group 2 subjects showed significant decrease in mean scores in all periodontal parameters as seen in Table 2. GI reduced to 0.5000 ± 0.52705 from 2.5000 ± 0.52705, PI to .6000 ± 0.51640 from 2.4000 ± 0.69921, PPD to 2.9000 ± 0.73786 from 5.900 ± 0.99443, CAL to 2.900 ± 0.56765 from 5.5000 + 0.52705 and mSBI to .8000 ± 0.42164 from 2.6000 ± 0.51640.

Table 4: Comparative evaluation of periodontal parameters at 3 months between groups

Variable	Groups	N	Mean	Std. Deviation	Mean difference	Student 't' test	P value
Gingival Index	Group 1	10	1.4000	.84327	.90000	2.862	.010*
	Group 2	10	.5000	.52705			
Periodontal Index	Group 1	10	1.3000	.67495	.70000	2.605	.018*
	Group 2	10	.6000	.51640			
Probing pocket depth	Group 1	10	4.1000	1.28668	1.20000	2.558	.020*
	Group 2	10	2.9000	.73786			
Clinical attachment loss	Group 1	10	4.2000	.78881	1.30000	4.230	.001*
	Group 2	10	2.9000	.56765			
mSBI	Group 1	10	1.4000	.51640	.60000	2.846	.011*
	Group 2	10	.8000	.42164			

*=Significant; NS = Not Significant

Periodontal parameters showed significant difference for Gingival index, Periodontal index, probing pocket depth, clinical attachment loss and mSBI at 3 months between Group A and Group B as seen in Table 4.

Table 5: Comparative evaluation of microbial count between groups

Microbes	Groups	N	Mean	Std. Deviation	Mean difference	Student 't' test	P value
P. Gingivalis - Baseline	Group 1	10	177.3000	11.37297	-2.80000	-.558	.583 (NS)
	Group 2	10	180.1000	11.04989			
A.actinomycetemcomitans – Baseline	Group 1	10	170.4000	7.16783	1.10000	.274	.787 (NS)
	Group 2	10	169.3000	10.45679			
P. Gingivalis - 3 months	Group 1	10	97.8800	10.94306	18.48000	3.429	.003*
	Group 2	10	79.4000	13.06565			
A.actinomycetemcomitans – 3 months	Group 1	10	115.4000	17.25109	34.20000	5.337	.000*
	Group 2	10	81.2000	10.63328			

*=Significant; NS = Not Significant

At baseline, both P.Gingivalis and A. actinomycetemcomitans had no significant difference between groups at p=0.583 and p=0.787. P.Gingivalis showed significant mean difference of 18.48000 at 3 months between groups at p=0.003. A. actinomycetemcomitans was found to be higher in Group 1 with a mean microbial count of 115.4000 X 10^3 while Group 2 had a mean of 81.2000 X 10^3 , which was significant at p=0.000 as seen in Table 5.

Data analysis:

Data was analysed using Statistical Package for Social Sciences (SPSS) 25.0 version (IBM; Chicago). One way Analysis of Variance (ANOVA) was applied to find significant difference at different time intervals of

baseline, 1 month and 3 months for Group 1 and Group 2. Student's t-test was run to find difference between groups at 3 months. For all analysis, level of significance was set at $p<0.05$.

All patients (12 males and 8 females with mean age of 35 ± 16 years) completed the study.

The age, sex and educational status were compared between the groups by using Pearson Chi-square test showing no statistical significance. GI, PPD, CAL and mSBI scores between the groups were similar at baseline and 1 and 3 months post-therapy [table 2 and 3].

GI within the groups at different time points was significantly ($P < 0.010$) different. Group 2 showed a significant reduction (to 0.5000 $\pm$ 0.52705 from 2.5000 $\pm$ 0.52705) than Group 1 (to 1.4000 $\pm$ 0.84327 from 2.6000 $\pm$ 0.51640) at the end of 3rd month. PPD and CAL within the groups at baseline 1 and 3 months, significantly reduced by 0.20 and .001 [table 4].

Microbiological assessment between the groups at different intervals showed no significance at baseline; however, statistically significant difference was observed at 3rd month post-treatment. [table 5]

Discussion

Chronic periodontitis is an infectious disease resulting in inflammation within the supporting tissues of the teeth , with progressive attachment and bone loss. [10]It is caused by mixed infections with the subgingival microbiota being organized as a biofilm and characterized by a continuous flux. [11] The interactions between the bacterial pathogenic microflora and the inflammatory responses of a susceptible host can produce the progressive destruction of periodontal tissues. [12] Nonsurgical mechanical periodontal treatment is the cornerstone of periodontal therapy and the first recommended approach to the control of periodontal infections.[13]The primary aim of non-surgical periodontal treatment is to arrest disease progression by eliminating bacterial infection, to reduce soft tissue inflammation and to reattach periodontal tissues to the root surface infected previously. [14]

Numerous clinical and microbiological studies have confirmed that non-surgical mechanical treatment consisting of plaque control and mechanical debridement, is effective in reducing the bacterial load, thus resulting in clinical improvement of the periodontal disease. [15,16] However, mechanical therapy itself may not always reduce or eliminate the anaerobic infection at the base of the pocket, within the gingival tissues, and in structures inaccessible to periodontal instruments. [17] Consequently, this led to the adjunctive use of antimicrobials, assuming that chemical aids would compensate for technical limitations and prevent early microbial recolonization to ultimately ensure the best chance for clinical improvements. [18]

Adverse effects such as drug toxicity, acquired bacterial resistance, drug interaction, and patients compliance limit the use of systemic antimicrobials.[19] Therefore, to override these shortcomings, local deliveries of antibacterial agents into periodontal pockets have been extensively studied [20]. This mode of drug delivery avoids most of the problems associated with systemic therapy, limiting the drug to its target site and hence achieving a much higher concentration. [21]

Chlorhexidine is an antimicrobial agent with a wide spectrum of activity encompassing gram-positive and gram-negative bacteria, yeasts, dermatophytes and some lipophilic viruses. However, subgingival irrigation using CHX solutions or even CHX gels turned out to be poorly effective in the treatment of periodontitis, presumably due to the inability to retain biologically significant concentrations of the drug for sufficient lengths of time within the confines of the periodontal pocket. [22] A biodegradable CHX chip for the controlled delivery of CHX to the periodontal pocket has been introduced.

Although studies exist comparing the efficacy of local drug delivery of

chlorhexidine chip on the treatment of periodontitis. Hence the present study was designed to compare the two non-surgical treatment modalities employed in the treatment of periodontitis.

In earlier studies CHX has shown good results when compared to SRP alone, but has shown several limitations in retention and application. Modification of Chlorhexidine delivery as slow releasing drug into the pocket has shown good results in treating periodontitis.[23,24,25,26] Chlorhexidine chip (DSI Sponge Perio, DSI Ltd, Ashdod, Israel) is a small, orange-brown in a rectangular chip form (rounded at one end) for easy insertion into periodontal pockets. Size of the chip is 4 x 5mm and thickness is 0.25-0.32mm and 10 mg weight. Each chip contains approximately 2.5 mg of CHX in a biodegradable matrix of fibrillar collagen of fish origin (DSI Sponge Perio, DSI Ltd, Ashdod, Israel). [27]

In the present study the efficacy of two non-surgical treatment modalities i.e. SRP and Chlorhexidine Chip in treating chronic periodontitis patients were assessed and analyzed.

The present study was undertaken to evaluate the efficacy of subgingivally placed controlled-release degradable chlorhexidine chip (DSI Sponge Perio, DSI Ltd, Ashdod, Israel) as an adjunct to scaling and root planing in the management of chronic periodontitis. Three months had been selected as the time duration of the study because effects of locally delivered controlled-release chlorhexidine have been shown to be evident up to 11 weeks after administration,[28,29] and 3 months correspond to the typical recall interval for periodontal patients.[30]

About two patients (20%) observed adverse effects like gingival pain and tender gums in group 2, whereas there was not even a single patient in group 1 who reported any side-effect due to scalling and root planning, the treatment procedure. Adverse effects occurring in the first week of the study appeared to be associated with chip placement at baseline after scaling and root planing. None of the changes discovered on oral examination were of a serious and irreversible nature.[31]

The comparison of clinical indices has shown reduction in PI scores in both the study groups. However, the highest reduction in PI scores was observed in group 2. The mean reduction of PI scores was 2.40 to 1.40 at 1 month and 0.60 at 3 month study intervals in group 2. The lowest reduction was observed in SRP group which were 2.20 to 1.20 at 1 and 1.30 at 3 months respectively. When reductions in PI scores were compared between the groups statistically significant difference was found between SRP group when compared with group 1 and group 2. Mean reduction in gingival index score was 0.5000 $\pm$ 0.52705 from 2.5000 $\pm$ 0.52705 in chlorhexidine chip group which was better than SRP group, which showed a mean reduction to 1.4000 $\pm$ 0.84327 from 2.6000 $\pm$ 0.51640 at 3 month interval, but these scores were statistically not significant. Chlorhexidine chip group showed statistically significant reduction in gingival scores at 1 month interval on comparison to SRP group. Similarly, studies by Soskolne et al. [23] , Heasman et al. [24] , Kasaj et al [25] . and Vishaka et al. [26] have shown significant reduction of plaque and gingival scores in chlorhexidine chip group when compared to SRP alone. The results of present study emphasize this reduction. Mean reduction in modified sulcular bleeding index scores was .8000 $\pm$ 0.42164 from 2.6000 $\pm$ 0.51640 in chlorhexidine chip group which was better than SRP group, which showed a mean reduction 1.4000 $\pm$ 0.51640 from 2.5000 $\pm$ 0.52705 at 3 month interval.

Among the two study groups, group 2 has shown highest reduction in PI, gingival index and modified sulcular bleeding index scores as evident in the results. The reduction in mean probing pocket depth was 2.9000 $\pm$ 0.73786 in group 2 at 3-month study interval. The intergroup comparison has shown that reduction in mean PPD scores in chlorhexidine group was statistically significant when compared with other groups. However, the reduction in PPD scores between SRP group and chlorhexidine chip group were statistically significant. Similarly, the mean reduction in CAL scores (2.900 $\pm$ 0.56765) were highest in group 2 and lowest (4.2000 $\pm$ 0.78881) in SRP group which were statistically significant.

Reduction in the gingival index score at baseline, 1 month and 3 months intervals was 2.60, 1.70, 1.40, respectively, for group 1 and 2.50, 1.00, 0.50, respectively, for group 2 as compared with baseline [table 2 and 3]. There was a statistically significant difference in the gingival index score observed at 3 months interval in group 1 and statistically highly significant difference in gingival index score at 1 month and at 3 months intervals in group 2 compared with baseline. These findings are in accordance with those of Azmak *et al.*, [32] who found a mean reduction in gingival index score at 1 month and 3 months for both the combination group and the scaling and root planning alone group when compared with baseline.

Chlorhexidine is an efficient antiplaque and antibacterial agent with wide spectrum. The application of chlorhexidine in locally delivered form in the form of chip has shown good results in the treatment of chronic periodontitis. [23,24,25,26]

Marjorie K Jeffcoat [30] has studied the effect of chlorhexidine chip on probing pocket depth and clinical attachment levels and found that the mean reduction in pocket depth was 0.85±0.12mm and mean reduction in clinical attachment levels was 0.92±0.17mm at 9 months. Similar results were reported by several other studies. [23,24,25,26]

The results of the present study emphasize this outcome with higher reduction rates in clinical indices gingival bleeding, PPD, CAL and mSBI scores.

In the present study, two treatment modalities compared and studied, chlorhexidine group showed significantly higher mean attachment gain of 2.900 $\pm$ 0.56765 at 3 month and statistically significant reduction in PPD scores. Thus results of the present study emphasize local delivery of chlorhexidine in the form of a chip was found to be efficacious in the successful treatment of chronic periodontitis.

Conclusion

In conclusion, the results of this study show that chlorhexidine chip containing 2.5 mg chlorhexidine

gluconate (DSI Sponge Perio, DSI Ltd, Ashdod, Israel) is an effective adjunctive therapy to scaling and root planing in the treatment of chronic periodontitis. It provides a safe, easily applied single-dose means of achieving significantly better clinical results than scaling and root planing alone. The adjunctive use of the chlorhexidine chip with scaling and root planing resulted in a clinically meaningful improvement in pocket depth reduction and clinical attachment level gain compared with scaling and root planing alone.

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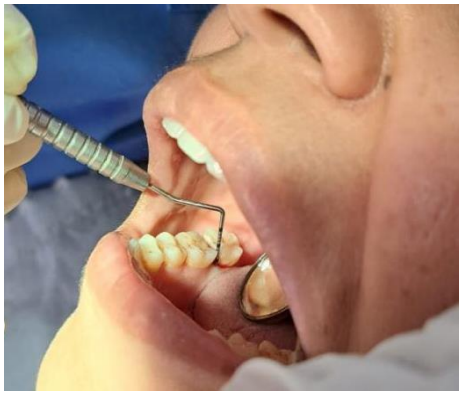
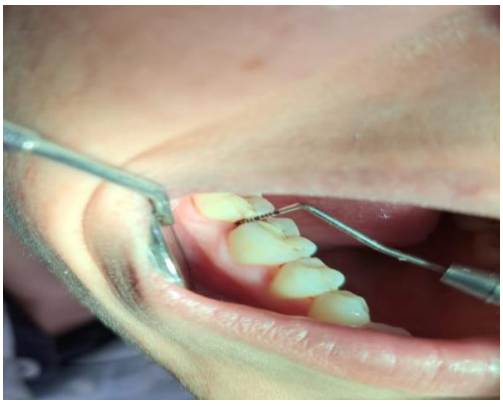

(A)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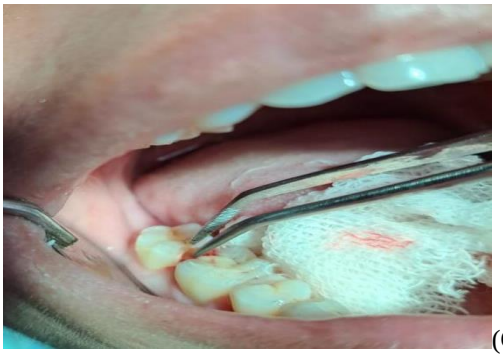
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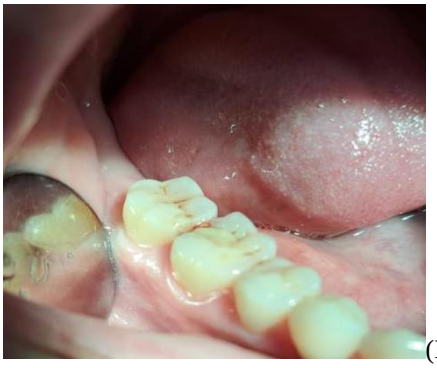
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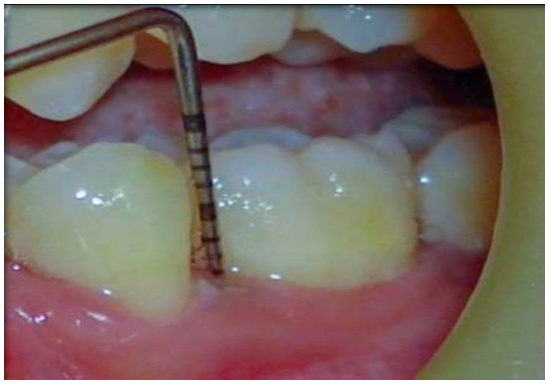
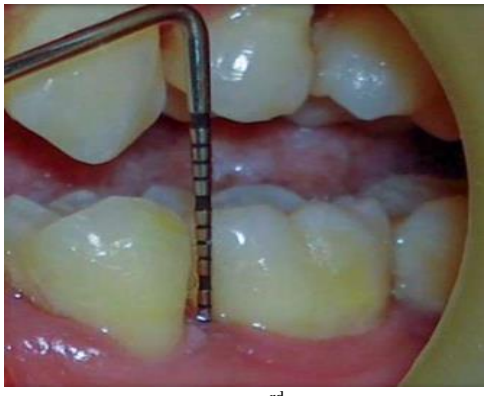
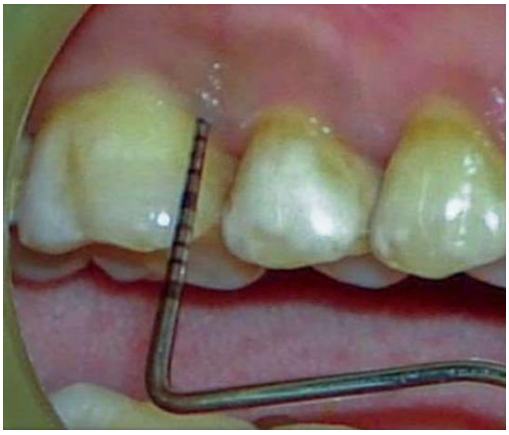


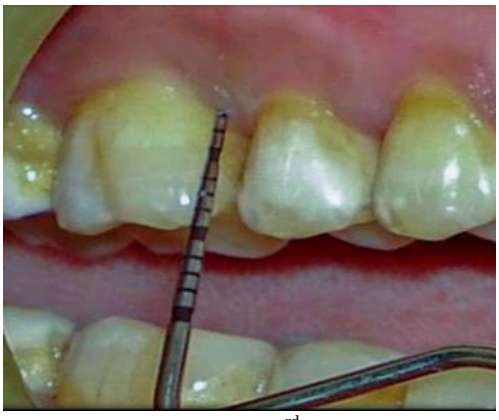
Group 1-probing depth at baseline



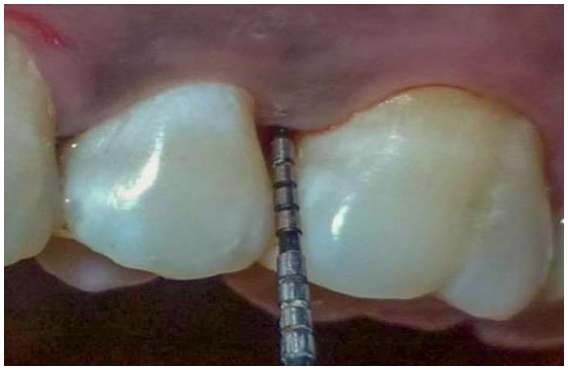
Group1- probing depth at end of 3rd month



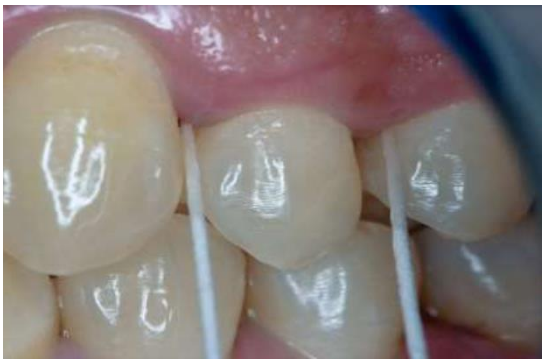
Group 2- probing depth at baseline



Group 2- probing depth at end of 3rd month



Selection of case based on ppd(>_ 5mm)



Collection of GCF sample

Chlorhexidine chip