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EVALUATION OF LOCALLY DELIVERED TETRACYCLINE IN MANAGEMENT OF CHRONC PERIODONTITIS: A MICROBIOLOGICAL STUDY

Dental Science

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ABSTRACT

Aim: To evaluate the efficacy of locally delivered antimicrobial agent tetracycline impregnated in collagen fibresin reducing the microbial load in deep periodontal pocket in terms of changing the clinical parameters of periodontal inflammation.
Material & Method: This study was performed on 974 sites from 40 patients. Out of these 40 patients, 5 patients from each study group (Total 15 patients) were randomly selected for microbiological analysis. The selected patients were evaluated for pre and post study microbiologic evaluation.
Result & observation: The pre and post study microbiological data were subjected to statistical analysis.
Discussion: This locally delivered antimicrobials were found to effective in reducing microbiological load in deep periodontal pockets.

KEYWORDS

Locally delivered antimicrobial, microbiological, deep periodontal pocket.

Introduction:
Periodontal diseases continue to be one of the most widespread afflictions of mankind. They comprise a group of inflammatory conditions of the supporting tissues of the teeth, that are initiated by microorganisms and resulting from the combined activity of microorganism and immunological response of the host.[1-5]The most common among the periodontal diseases is chronic periodontitis that occurs as an infection caused by microorganisms, predominantly anaerobic bacteria, particularly spirochetes and gram negative rods.[7-9]In chronic periodontitis, the bacteria most often cultivated at high levels include *P. Gingivalis, B. forsythus, P. Intermedia; C. Rectus, Eikenellacorrodens, F. nucleatum,A. actinomycetemcomitans, P. micros, and Treponema and Eubacterium* spp.[10].

Total plaque control is therefore considered an important step in prevention and treatment of inflammatory periodontal disease.

However, the patients who have demonstrated periodontal disease progression despite supportive periodontal therapy, showed elevated level of putative periodontal pathogens in subgingival plaque; which include *Bacteroides forsythus, Fusobacterium nucleatum, Streptococcus intermedius, Eikenella corrodens. And Porphyromonasgingivalis.*[9,10] Thus failure of conventional periodontal therapy may also be related to an insufficient suppression or incomplete elimination of periodontopathic bacteria or even the penetration within the gingival connective tissue. Due to the presence of persistent bacteria, antimicrobials are currently regarded as a first choice for adjunctive therapy of periodontitis.[8-10]Tetracycline offers a broad-spectrumantimicrobial activity and may be a useful adjunct to periodontal therapy.Local delivery of tetracycline can maintain high antibiotic concentration in the gingival crevicular fluid over a period of 10-14 days.[11]]

Therefore, the use of local delivery of antimicrobials, particularly tetracycline, as an adjunct in the treatment of periodontitis has received considerable attention during the past. Therefore, the present study has been performed to evaluate the microbiological efficacy of local delivery of tetracycline impregnated in collagen fibres in the management of chronic periodontitis in terms of reducing the microbial load.

Aims & Objective:
The study was undertaken to test the efficacy of local drug delivery of antimicrobial, namely tetracycline, impregnated in collagen fibre mesh (of Type I collagen Periodontal Plus – AB) within the periodontal pocket with the following aims:
I. to find out the common bacteria (anaerobic) in chronic periodontitis.
ii. quantization of the bacteria associated with chronic periodontitis.
iii. to find out the efficacy of local drug delivery (tetracycline impregnated in collagen fiber) in reduction of periodontopathic pathogen.

Material& Methods:
The study was done in accordance with the clearance obtained from the Institutional Ethics Committee. The local drug delivery of tetracycline (Periodontal Plus -AB) is available in strips containing four individually packed and separable sterile product pocket, containing approximately 1.7 ± 0.25 mg of tetracycline HCL.

This study was performed on 974 sites from 40 patients selected for the study.

a. Inclusion Criteria:
(i) Patients were of either sex in the age group of 25-70 years.
(ii) Pocket depth of 5 mm and above in any surface of the tooth was included in the study.
(iii) Subjects who should not have received any antibiotic therapy for last one month.

b. Exclusion Criteria:
(i) Patients who were medically compromised.
(ii) Patients allergic to tetracycline and/or Chlorhexidine.
(iii) Pregnant and lactating women.
(iv) Patients showing unacceptable oral hygiene even after motivation.

All patients participating in this study were explained regarding the treatment plan and its possible outcome and a written consent was obtained from each of them.

The study model consisted of three groups namely,A, B & C. Selected subjects were randomly allocated to any of the above groups such as follows

1. Group A – Control Group [treated with scaling and root planing only].

2. Group B – First experimental Group (No. 1) [treated with scaling, root planing plus tetracycline insertion in the pocket].
3. Group C – Second experimental Group (No. 2) [treated with only tetracycline insertion].

Microbiological analysis of plaque sample:
A sum total of fifteen number of patients were randomly allocated for microbiological analysis. After 1st day visit measurement, the deepest periodontal pockets were selected for the microbiological sample sites. In post treatment phase after 60 days measurements, again plaque sample were collected from the same site. To avoid the use of transport media, all the procedures were done in the anaerobic laboratory of the department. Supragingival plaque was removed with a sterile cotton piece prior to sample collection. Following removal of supragingival plaque, sub-gingival plaque was collected with a 30 No. paper point after 30 seconds of insertion. The sample were transferred into 1.9 ml of sterile normal saline by vigorously agitating tip of the paper point into the solution because the absorption capability of paper point 0.1 ml for making the solution 2 ml. The Bacteria were dispersed with a vortex mixer for 60 seconds to minimize the clumping. Then the samples were inoculated in brain heat infusion agar after preparing five different culture media by addition of different antibiotic to make the bacteria selective for genus level isolation.To enhance the growth, isolation, and selection of etiologic agents, specimen inocula are usually spread over the surface of plates in a standard pattern so that individual bacterial colonies are obtained and semi-quantitative analysis can be performed.The inoculated plates were immediately incubated under anaerobic conditions at 35ºC to 37ºC for 72 hours in an anaerobic gas pack jar.

From the plates of anaerobic jar the colonies were screened after the expiry of incubation period with the help of 10X lens. The representative colonies were subcultured aerobically (in blood Agar Plate) and anaerobically for 48 hours. The aero tolerance colonies were considered as fucultative anaerobe. On the other hand, the colonies growing anaerobically but not under aerobic conditions were regarded as obligate anaerobes.

Genous level identification of the common periodontopathic bacteriae was done on the basis of –

A. Growth in a selective media.
B. Colony Character.
C. Gram stain morphology

Results & Observations:
40 patients, in the age range of 25-70 years provided 974 sites for the present study. Out of 40 patients, 15 patients were subjected to microbiological analysis. All patients were randomly allocated in 3 categories, namely A, B, C. All patients who were included in this study showed biocompatibility of the material used, that is Periodontal Plus – AB.

As regards microbial flora pretreatment and post-treatment level which shows an array of anearobic microbial flora predominantly consisting of *Peptococcus, Peptostreptococcus, Prevolella, Porphyromonas. Bacteroides, Fusobacterium, Viellonella, Lactobacillous and Actinomyces.*

1. Gm-ve small cocco-bacilli rods (Genera : Bacteroids, Prevotella, Porphyromonas) : (Table-1, Fig.1)
At pre-treatment stage magnitude of anaerobic gm-ve small cocco-bacilli rods was 10.816 0.803 in category A, 10.991 0.020 in category B and 10.762 0.454 in category C, which shows no statistically significant difference (p>0.05) in between categories.

On 60 days after treatment flora shows declining trend 8.676 0.075 in category A, 4.276 0.616 in category B and 6.528 0.848 in category C. Log values of mean difference between pre and post treatment shows reduction of gm-ve small cocco-bacilli by 2.14, 6.715 and 4.234 for category A, B and C respectively. Comparison between groups A Vs. B; A Vs. C and B Vs. C at post-treatment level shows statistically significant t_8 values (7.32, 3.58 and 3.256 respectively).

Corresponding P values were P<0.001, P<0.01 and P<0.05.

2.Gm+ve long stout rods (Table 2; Fig. 2) :
At pre-treatment stage magnitude of anaerobic gm-ve long stout rods was 10.982 0.025 in category A, 11.172 0.463 in category B and 10.543 0.541 in category C which shows no statistically significant difference (P>0.05) in between category.

On 60 days after treatment flora shows declining trend 9.279 0.884 in category A, 4.102 0.450 in category B and 6.370 0.624 in category C. Log values of means difference between pre and post treatment level shows reduction of gm+ve long stout rods were by 1.703, 7.07 and 4.173 for category A, B and C respectively. Comparison between groups A Vs. B; A Vs. C and B Vs. C at post-treatment level shows statistically significant t_8 values (6.05, 4.63 and 3.34 respectively). Corresponding P values were P<0.001, P<0.01 and P<0.05.

3.Gm-ve slender filamentous body with L branching (Genus Actinomyacetes (Table 3, Fig. 3)
At pre-treatment stage magnitude of anearobic gm-ve slender filamentous body with L branching was 10.791 0.443 in category A, 10.782 0.438 in category B and 10.734 0.465 in category C which shows no statistically significant difference (P>0.05) in between category.

On 60 days after treatment flora shows declining trend 9.111 0.577 in category A, 3.835 0.491 in category B and 6.471 0.911 in category C. Log values of means difference between pre and post treatment level show reduction of magnitude by 1.68, 6.95 and 4.263 for category A, B and C respectively.

Comparison between groups A Vs. B; A Vs. C and B Vs. C at post treatment value shows statistically significant t_8 values were 16.47, 5.90 and 6.21 respectively. Corresponding P values were P<0.001; P<0.001 and P<0.001.

4.Gm-ve cocci (Veillonella) (Table 4) :
At pretreatment stage magnitude of anearobic gm-ve cocci was 11.173 0.463 in category A, 10.772 0.459 in category B and 10.553 0.548 in category C, which shows no statistically significant difference (P>0.05) in between category.

On 60 days after treatment flora shows declining trend 9.436 0.496 in category A, 4.267 0.596 in category B, 6.105 0.587 in category C. Log values of means difference between pre and post treatment stage show reduction of anearobic gm-ve cocci by 1.737, 6.505 and 4.448 for category A, B and C respectively.

Comparison between groups (A Vs. B), (A Vs. C) and (B Vs. C) at post treatment level shows statistically significant t_8 values were 11.07, 6.28 and 3.82 respectively corresponding P values were P<0.001, P<0.001 and P<0.01.

5.Gm-ve fusiform rods (Table 5):
At pre-treatment stage magnitude of anearobic gm-ve fusiform rods was 10.808 0.707 in category A, 11.182 0.458 in category B and 10.563 0.556 in category C, which shows no statistically significant difference (P>0.05) in between category.

On 60 days after treatment, flora shows declining trend 8.972 0.459 in category A, 4.241 0.541 in category B and 5.96 0.83 in category.

Log values of mean differences between pre and post treatment stages show reduction of gm-ve fusiform rods were by 1.836, 6.941 and 4.60 for group A, B and C respectively.

Comparison between groups A Vs. B; A Vs. C and B Vs. C at post-treatment level shows statistically significant t_8 values were 8.35, 3.39, 4.31 respectively.

Corresponding P values were P<0.001, P<0.01 and P<0.01.

6.Gm+ve cocci in short chain (Peptococcus, Peptostreptococcus) (Table 6) :
At pretreatment stage magnitude of anearobicgm+ve cocci in short chain were 10.973 0.025 in category A, 10.562 0.557 in category B and 10.522 0.567 in category C, which showed no statistically significant difference (P>0.05) in between category.

On 60 days after treatment flora shows declining trend 9.592 0.417 in category A. It showed increase level of 10.777 0.441 in category B and 10.961 0.053 in category C.

Log values of mean differences between pre and post-treatment stages showed reduction of gm+ve cocci in short chain was 1.381 in category A. Some was increased level in category B – 0.215 and – 0.439 in category C.

Comparison between groups A Vs. B, A Vs. C shows statistically significant t_8 values were 5.55 and 6.05 respectively. Corresponding P values were P<0.001 and P<0.001. The same values for B Vs. C showed no significance (P>0.05).

Discussion:

In this study the microbiological parameters of catagory A, B and C were compared from baseline to the 60 days post-treatment period. The samples were collected from deepest periodontal pockets in the selected 15 patients, with the help of sterile paper point. Following 60 days post-treatment evaluation, results were obtained in terms of gm-ve small cocco-bacilli rods, gm+ve long stout rods, gm-ve slender filamentous body. gm-ve cocci, gm-ve fusiform rods and gm+ve cocci in short chains. The result showed that, the initial magnitude of above mentioned bacteriae for all category were more or less same, which showed no statistically significant difference (P>0.05) between the categories. In the case of gm-ve small cocco-bacilli, the difference between pre and post treatment magnitude were reduced in all category namely A (P<0.05), B (P<0.001) and C (P<0.001). Comparison between groups at post-treatment level showed statistically significant difference A Vs. B was P<0.01, A Vs. C was P<0.01 and B Vs. C was P<0.05.The result showed that all bacteriae at post-treatment level statistically significantly reduced. Category B set showed the best treatment for chronic periodontitis, followed by C and A. Comparison between categories in post-treatment level showed highly significant statistically difference [A Vs. B P<0.01, A Vs. C P<0.001]. B Vs. C showed statistically not significant (P>0.05). Similar results were also found by several researchers.[12-14] Although several authors were quantitatively determined the levels of *A. actinomycetemcomitans, E. corrodens, F. nucleatum, P. gingivals, P. intermedia* and *V. recta* from subgingival plaque samples by DNA probe method.[15] This method is more specific and sensitive than culture. In this multicentre study the effect of tetracycline fiber therapy were compared with control fiber, scaling and root planing and untreated sites. Both locally delivered tetracycline fiber therapy and scaling decreased the no. of sites infected with all the monitored species. The percentage of reduction of the no. of sites with detectable infection varied with each species from 86% W. recta to appx. 40% *P gingivalis*. Significant reduction of pocket depth and bleeding occurred at TC fiber treated sites infected with each of the species. Significant attachment level gain occurred only at sites initially infected with *P. gingivalis.*

As this preparation is time limiting and there was constraints as regards availability of very good anearobic technology, we may have missed many other anearobes likely to be present in this situation.This include several oral cultivable Treponema, Eubacterium, and very fastidious and remarkable oxygen sensitive non clostidial gm-ve anearobes. Species level identification was beyond the scope of this investigator for lack of gas liquid chromatography in the laboratory. Still a genus level identification of bacteria and their quantitation is good enough to evaluate rough estimation of different modes of management on the problem of chronic periodontitis.

Conclusion:

As the research method is time limiting and there was constraints as regards availability of very good anaerobic technology, we may have missed many other anearobes likely to be present in this situation. This include several oral cultivable Treponema, Eubacterium, and very fastidious and remarkable oxygen sensitive non clostidial gm-ve anearobes. Species level identification was beyond the scope for lack of gas liquid chromatography in the laboratory. Still a genus level identification of bacteria and their quantization is good enough to evaluate rough estimation of different modes of management on the problem of chronic periodontitis.

Fig 1: Cylinder diagram showing magnitude of gram-ve Fusiform rods population in chronic periodontitis

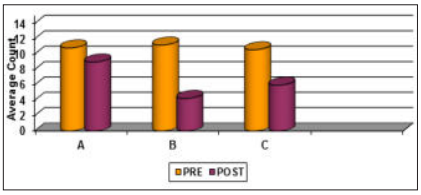

Figure 2: Bar diagram showing magnitude of gram+ve long stout rods population in chronic periodontitis

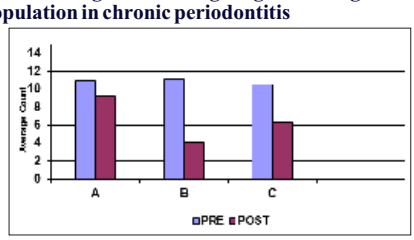

Figure 3: Bar diagram showing magnitude of gram-ve slender filamentous body with L branching population in chronic periodontitis

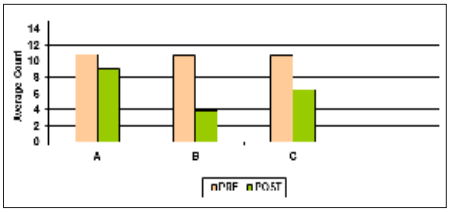

Table1. Gm-ve small cocco-bacilli average count (after Log. transformation) with S.D. value on pre & post 60 days period for samples under three groups and test of significance after differences

	AV ± SD			Significance of the differences in averages between categories		
	A	B	C	A Vs. B	A Vs. C	B Vs. C
Pre	10.816 ± 0.803	10.991 ± 0.020	10.762 ± 0.454			
Post	8.676 ± 0.075	4.276 ± 0.616	6.528 ± 0.848			
Pre-Post	d = 2.14;	d = 6.715;	d = 4.234;			

Table 2: : Gram+ve long stout rods average count (after Log. transformation) with S.D. value on pre & post 60 days period for samples under three groups and test of significance after differences

	AV ± SD			Significance of the differences in averages between categories		
	A	B	C	A Vs. B	A Vs. C	B Vs. C
Pre	10.982 ± 0.025	11.172 ± 0.463	10.543 ± 0.541			
Post	9.279 ± 0.884	4.102 ± 0.450	6.370 ± 0.624			
Pre-Post	d = 1.703;	d = 7.07;	d = 4.173;			

Table 3: Gram-ve slender filamentous body with L branching average count (after Log. transformation) with SD value on pre & post 60 days period for samples under three groups and test of significance after differences

	AV ± SD			Significance of the differences in averages between categories		
	A	B	C	A Vs. B	A Vs. C	B Vs. C
Pre	10.791 ± 0.443	10.782 ± 0.438	10.734 ± 0.465			
Post	9.111 ± 0.577	3.835 ± 0.491	6.471 ± 0.911			
Pre-Post	d = 1.68;	d = 6.95;	d = 4.263;			

Table 4:Gm-ve cocci average count (after Log. transformation) with SD value on pre & post 60 days period for samples under three groups and test of significance after differences.

	AV ± SD			Significance of the differences in averages between categories		
	A	B	C	A Vs. B	A Vs. C	B Vs. C
Pre	11.173 ± 0.463	10.772 ± 0.459	10.553 ± 0.568			
Post	9.436 ± 0.496	4.267 ± 0.596	4.105 ± 0.587			
Pre-Post	d = 1.737;	d = 6.505;	d = 6.448;			

Table 5: Gm-ve fusiform rods average count (after Log. transformation) with S.D. value on pre & post 60 days period for samples under three groups and test of significance after differences

	AV ± SD			Significance of the differences in averages between categories		
	A	B	C	A Vs. B	A Vs. C	B Vs. C
Pre	10.808 ± 0.707	11.182 ± 0.458	10.563 ± 0.556			
Post	8.972 ± 0.459	4.241 ± 0.541	5.96 ± 0.83			
Pre-Post	d = 1.836;	d = 6.941;	d = 4.60;			

Table 6: Gm+ve cocci with short chain average count (after Log. transformation) with SD value on pre & post 60 days period for samples under three groups and test of significance after differences

	AV ± SD			Significance of the differences in averages between categories		
	A	B	C	A Vs. B	A Vs. C	B Vs. C
Pre	10.973 ± 0.025	10.562 ± 0.587	10.522 ± 0.567			
Post	9.592 ± 0.417	10.777 ± 0.441	10.961 ± 0.53			
Pre-Post	d = 1.381;	d = -0.215;	d = -0.439;			

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