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"A COMPARATIVE EVALUATION OF TETRACYCLINE CONTAINING MICROSPHERES AND COMMERCIALLY AVAILABLE TETRACYCLINE FIBERS TO EVALUATE THEIR EFFICACY IN PERIODONTAL POCKET THERAPY - A CLINICAL AND MICROBIOLOGICAL STUDY"

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ABSTRACT

The aim of this clinical trial was to compare the efficacy of Tetracycline containing Microspheres (TM) and commercially available tetracycline containing Fibers (TF) as local drug-delivery system in the treatment of periodontal pocket therapy. A randomized split mouth design was adopted for allocating, 50 sites in different treatment modalities : SRP + TF and SRP + TM. Periodontal Plus AB was used for TF group and prepared microsphere containing Tetracycline was used for TM group. Clinical parameters were seen at baseline, 1 and 6 months and microbiological analysis was done at baseline and 3 months. Clinical parameters that were recorded were PI, GI, PD and RAL. In microbiological analysis, CFU of Prevotella Intermedia were seen. The result revealed that both groups showed a statistically significant reduction in clinical parameters and microbiological parameters at 1, 3 and 6 months compared to baseline and TM was more effective in comparison to TF.

KEYWORDS : Tetracycline Microspheres, periodontal pocket therapy, prevotella intermedia

INTRODUCTION

Traditional scaling and root planing (SRP), although the cornerstone of periodontal therapy, often leave behind a significant number of pathogenic bacteria, and the recolonization of the same bacteria occurs in as early as 60 days.[1] So in cases where patients are unresponsive or marginally unresponsive to SRP, the adjunctive use of chemotherapeutic agents, either systemically or locally extends the effects of therapy.[2-4].

Systemic administration has the advantage of the concurrent treatment of entire oral cavity. However, it's disadvantages include higher risk of toxicity, chances of superinfection and acquired bacterial resistance[5].

In order to overcome these problems, local drug delivery was selected as mode of drug delivery using tetracycline drug.

The aim of the study was to comparatively evaluate the efficacy of commercially available Tetracycline fibers (Periodontal Plus AB) and Tetracycline containing Microspheres in treatment of chronic periodontitis patients with the help of clinical and microbiological aids.

MATERIAL AND METHODS

The following Clinical - Microbiological study was carried out in the Department of Periodontics, Babu Banarasi Das College of Dental Sciences, Lucknow, in collaboration with Department of Pharmacy, B.B.D.N.I.I.T. , Lucknow and Department of Microbiology, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow. The current study was executed after receiving an appropriate clearance from the Institutional Ethics Committee.

This was an interventional, prospective, randomized split-mouth clinical and microbiological study. After giving a brief description of the study to the patient, a consent form was duly signed by the patient. The selection of volunteers was based upon the following inclusion and exclusion criteria:

INCLUSION CRITERIA: 1)Chronic periodontitis patients with sites of probing depth (PD) ≥ 4 mm. 2)Systemically healthy patients. 3)Patients who have not undergone any invasive periodontal therapy in the past 6 months.

EXCLUSION CRITERIA: 1)Patient suffering from Aggressive Periodontitis. 2)Patients having Teeth with ≥ grade II furcation (Glickman's classification). 3)Patients who have received antibiotics and anti-inflammatory drugs within the preceding 3 months of the study. 4)History of known allergy to Tetracycline. 5)Smokers and/or tobacco chewers. 6) Pregnant and lactating women.

STUDY DESIGN -

A total of 50 sites were included in the study which were randomly and equally divided into following two groups -

1.TETRACYCLINE FIBER (TF) GROUP - There were 25 sites in this group and each participant in this group was treated with 10 mg of accurately weighed commercially available TF (Periodontal Plus AB) which was equivalent to 1 mg of drug

2.TETRACYCLINE MICROSPHERE (TM) GROUP - There were 25 sites in this group and for each patient 0.2 ml of prepared TM equivalent to 1 mg of drug was used, which was given with the help of 1 ml syringe and a blunt canula attached to it.

PREPARATION OF TETRACYCLINE CONTAINING MICROSPHERE AND SEM ANALYSIS

Tetracycline containing microsphere (TM) were prepared using Tetracycline and ethyl cellulose (polymer). After preparation of TM it was converted into thermoreversible gel (0.5 % W/V) with gelation temperature at 32.66°C. Before converting TM into thermosreversible gel, the sample was sent for SEM analysis to check for loading of the drug. Images of blend microspheres, with tetracycline loaded and after drug release were obtained with an SEM.

Fig 1 - Prepared tetrcycline microspheres

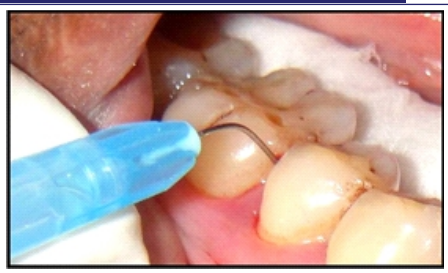

Fig 1.1 - use of blunt canula for LDD

DRUG CONTENT

Accurately weighed tetracycline microsphere (100mg) were mechanically powdered and amount of drug entrapped was calculated using UV spectrometer at 275 nm after suitable dilution. Entrapment efficiency was found to be 92.6% and drug loading was found to be 23.15%.

The following clinical parameters were recorded at baseline, 1 months and 6 months in the Case-history proforma -

1. Plaque Index[6]
2. Gingival Index[7]
3. Probing Pocket Depth (PPD)
4. Relative Attachment Level (RAL)

MICROBIOLOGICAL ANALYSIS - Microbiological analysis was done for Prevotella Intermedia species quantitively by counting the colony forming units at baseline and after 3 months.

After recording the clinical parameters, plaque sample was collected from the selected sites with the help of gracey curette and was immediately transferred to the transport media (Luriabertani). The plaque sample was inoculated in duplicate on Wilkinson's Anaerobic Blood Agar Media, and Selective Media for Prevotella Intermedia separately. Culture plates were incubated under strict anaerobic environment in Macintosh anaerobic jar . One set of culture plates (of both Wilkinson's Blood Agar Media and Selective Media for Prevotella Intermedia) was incubated for 3 days and the other duplicate set was incubated for 14 days. After incubation, growth was identified on the basis of their colony characteristics and morphology. Confirmation of Prevotella Intermedia isolate was done by VITEK-2(Automated ID system).Colony forming units of Prevotella Intermedia was counted with the help of automated colony counter by Schuett Biotech. dc colony quant.

Data and Statistical Analysis

The principal clinical and microbiological findings of the study were plaque index (PI), gingival index (GI), pocket probing depth (PPD), relative attachment level (RAL), and bacterial count (BC) of *Prevotella Intermedia*. Data were summarized as Mean ± SD (standard deviation). Groups were compared by two factor repeated measures analysis of variance (ANOVA) and the significance of mean difference within (intra) and between (inter) the groups was done by Tukey's post hoc test after ascertaining normality by Shapiro-Wilk's test and homogeneity of variance between groups by Levene's test. A two-tailed p value less than 0.05 ($p<0.05$) was considered statistically significant. All analyses were performed on SPSS software (windows version 17.0).

RESULTS

I)Plaque index (PI) - For TF group PI was 1.574 ± 0.122 at baseline which decreased to 1.240 ± 0.138 at 1 month, which further increased to 1.347 ± 0.138 at 6 months. For TM group PI was 1.582 ± 0.188 at baseline which decreased to 1.075 ± 0.136 at 1 month and increased to 1.219 ± 0.156 after 6 months.

II) Gingival index - For TF group GI was 1.386 ± 0.140 at baseline which decreased to 1.174 ± 0.138 at 1 month, which further increased to 1.274 ± 0.124 at 6 months. For TM group GI was 1.404 ± 0.165 at baseline which decreased to 1.024 ± 0.169 at 1 month and increased to 1.134 ± 0.168 after 6 months.

III.Pocket Probing depth -

Table 5: Pre and post PPD (Mean ± SD, 25) of two groups

Periods	Fiber	Microsphere	p value
Baseline	4.716 ± 0.338	4.871 ± 0.336	0.554
1 month	3.602 ± 0.339	3.312 ± 0.318	0.005
6 month	3.586 ± 0.337	3.234 ± 0.295	0.032

Table 6: Intragroup comparison (p value) of mean PPD at different time intervals by Tukey test

Comparisons	Fiber	Microsphere
Baseline vs. 1 month	<0.001	<0.001
Baseline vs. 6 month	<0.001	<0.001
1 month vs. 6 month	0.991	0.041

IV.Relative attachment level -

Table 7: Pre and post RAL (Mean ± SD, 25) of two groups

Periods	Fiber	Microsphere	p value
Baseline	5.672 ± 1.061	5.574 ± 1.076	0.999
1 month	4.492± 0.850	4.384± 0.829	0.014
6 month	4.485± 0.965	4.028 ± 1.011	0.040

Table 8: Intragroup comparison (p value) of mean RAL at different time interval by Tukey test

Comparisons	Fiber	Microsphere
Baseline vs. 1 month	<0.001	<0.001
Baseline vs. 6 month	<0.001	<0.001
1 month vs. 6 month	0.096	0.921

V.Bacterial count -

Table 9: Pre and post bacterial count (Mean ± SD, 25) of two groups

Periods	Fiber	Microsphere	p value
Baseline	63998 ± 44473	64684 ± 48091	1.000
3 month	27624 ± 20263	1178 ± 2954	0.039
p value	<0.001	<0.001	-

Statistical analysis demonstrated a significant ($p<0.001$) decrease in clinical parameters, plaque index & gingival index in both TF group & TF group at 1 months & 6 months from baseline. However, in both the groups, PI and GI increased at 6 month as compared to at 1 month but the increase was not significant ($p>0.05$). Intergroup comparison of PI and GI between the groups showed no difference at baseline. However, at both 1 month and 6 month, PI and GI lowered significantly in TM than TF. PPD lowered significantly ($p<0.001$) at 1 and 6 month as compared to baseline in both the groups. However, at 6 months when compared to 1 months, PPD decreased insignificantly ($p>0.05$) for TF but the decrease for TM was significant ($p<0.05$). Intergroup comparison of PPD showed statistically lower ($p<0.05$) at 1 months and ($p<0.01$) at 6 months in TM. there was significant decrease ($p<0.001$) in RAL in both groups at 1 months and 6 months as compared to baseline, while not differed statistically ($p>0.05$) between 1 months and 6 months. Intergroup comparison of RAL at both 1 and 6 months, showed significant ($p<0.05$) decrease in TM than TF.

In microbiological analysis, bacterial count of prevotella intermedia at 3 months decreased significantly ($p<0.001$) in both groups as compared to baseline. In intra group analysis, at 3 month, it decreases significantly ($p<0.001$) in TM in comparison to TF. Decrease was 31.4 % higher in TM(98.2%) in relation to TF(56.8%).

DISCUSSION - Since 1979, attention has been given to delivering an antibiotic to specific site of periodontal infection.[9] Both these treatment modalities, SRP and local drug delivery, work on different principle, whereas SRP acts by removing bacteria and calculus deposits, local drug delivery acts by its bactericidal or bacteriostatic action. Therefore, neither is the ideal control of the other and adjunctive use of local drug delivery along with SRP gives cumulative effect in treatment of chronic periodontitis.[10]

Owing to the limitations of TF as local drug delivery agent there is need to look for other agents for local drug delivery.

In the second group of our study, Tetracycline containing microspheres were used. Microspheres are small spherical particles, with diameters in the range of 1 - 1000µm. This is an important approach in delivering therapeutic substances to the target site. Microsphere morphology allows the drug to be released and degraded in a sustained and controlled mode. It reduces the dosing frequency and thereby improves the patient compliance. Due to its spherical shape and smaller size the drug is easily placed in the sites. Bioavailability of the drug is improved because of better drug utilization. Incidence and intensity of adverse effects is also reduced.[11] According to available literature, when a drug is loaded in microsphere, there is initial release of drug from the surface of microsphere within the first twenty-four hours, which is known as burst release and the rest of the drug is released in sustained manner over a period of approximately 14 days.[12] All these advantages of microspheres as drug delivery system, has led to its use in different fields of medical sciences like in ophthalmic, nasal and vaginal drug delivery. It is in dentistry only that uptil now there has not been much use of microspheres as drug delivery system.

TM was prepared in liquid form so that it can be easily placed into the periodontal pocket with the help of blunt canula. Normal body temperature is 36.7^{0}C and the gelation temperature of TM was 32.66^{0}C. So, when TM was placed inside periodontal pocket it got converted into gel form, helping in better stabilization of the drug at the affected sites.

There was decrease in PI and GI at 1 and 6 months which can be attributed to SRP done at baseline, proper oral hygiene maintenance and patient motivation. The decrease can also be attributed to Hawthorne effect, in which individuals modify or improve an aspect of their behaviour in response to their awareness of being observed. Another important factor was the use of tetracycline as antimicrobial agent , that is effective against, both aerobic and anaerobic, gram-positive and gram-negative cocci and bacilli, found in the oral cavity. Anti - inflammatory properties of the tetracycline[13,14] also contribute to reduction of inflammation. All these factors helped in attaining good periodontal health. When comparing between 1 and 6 months, PI and GI increased at 6 months, but, it was still lesser than at baseline. The increase in PI and GI could be due to the fact that drug has no anti plaque properties as such and negligence shown by patient towards oral hygiene.

Probing depth was significantly reduced after 1 months in both the groups, which could be due to reduced inflammation and better healing in connective tissue adjacent to junctional epithelium. Another reason for this can be due to the reduction in subgingival microflora which is again attributed to the use of tetracycline along with mechanical debridement. At 6 months although, there was not significant decrease in probing depth for both the groups, but it was still lesser than that at 1 months which shows that there was still continuous improvement.

At 1 months, Relative attachment level decreased significantly for both groups. This could be attributed to the non-antibiotic properties of Tetracycline namely anti-collagenase[15,16] and anti-inflammatory effect along with the effect of mechanical debridement. Other properties of tetracycline, like, periodontal regenerative properties and its ability to remove smear layer from cementum[17], also helps in better attachment. Also, at 6 months, there was still decrease in RAL in comparison to 1 months which indicates that the effect of drug was still present .

When intergroup comparison was done for all the clinical parameters, TM showed better results in comparison to TF at both 1 and 6months. This can be attributed to the type of vehicle used for delivering the drug. Microsphere has a property of sustained drug release over a longer duration of time. The drug is stored inside the microspheres, and it is released slowly and gradually over a period of time. Also, microspheres are very tiny particles which could easily get absorbed into mineralized dental structures, where it may act as a transient reservoir of the antimicrobial agent.[18]

In microbiological analysis, both groups were able to significantly reduce the CFU of Prevotella Intermedia at the end of 3 months. But, when comparing the two drugs it was seen that decrease was 31.4% higher in microsphere(98.2%) in relation to fiber(56.8%). Thus, microbiological analysis also gave superior results with TM in comparison to TF.

CONCLUSION -

So far, there has not been any comparative evaluation done between tetracycline fiber and tetracycline microsphere in treatment of chronic periodontitis (CP). Therefore, on the basis of this study, it may be concluded with the help of clinical parameters and microbiological analysis that TM were more efficient than TF in treatment of chronic periodontitis. Also, TM was better mode of local drug delivery in comparison to TF for both dentist and patient in terms of usage and application.

However, further studies need to be done with larger sample size, longer study duration and multivariate microbiological species in order to reinforce the superiority of TM over TF.

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