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# Effect of Tetracycline Hydrochloride Fibers (Periocol-Tc) on The Level of P. Gingivalis in Chronic Generalized Periodontitis: Clinical & Microbiological Study

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***Abstract***

***Aim:*** *To compare the efficacy of locally delivered drug (LDD) tetracycline hydrochloride fibers (Periocol- TC) on the levels of Porphyromonas gingivalis (P.g) when used as an adjunct to scaling and root planing (SRP) in the treatment of chronic generalized periodontitis.*

***Materials &Methods****: 50 patients (both males and females) with 100 periodontitis sites were selected in the age group of 30– 55 years. All sites were randomly assigned to experimental or control group (50 sites in each group). Experimental sites (Group A) were treated with SRP and Periocol-TC. Controls (Group B) were treated with SRP alone. Plaque samples were collected and clinical parameters of Plaque Index(PI), gingival index(GI), periodontal pocket depth (PPD) and relative attachment level (RAL) gain were recorded on baseline, 15th and 45th day for quantitative and qualitative analysis of P g.*

***Results:*** *There was a statistically significant improvement in all variables for both the groups. However in experimental group; reduction in PI, GI, PPD and colonies of Porphyromonas gingivalis ; and gain terms of RAL, were statistically more as compared to control group.*

***Conclusion:*** *LDD, Periocol – TC may be a valuable adjunct to SRP in the Treatment Of Chronic Periodontitis.*

***Keywords****: Local drug delivery, Porphyromonas gingivalis, Tetracycline fiber (Periocol-TC), Thioglycollate culture media*

## I. Introduction

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by specific microorganism and their end products causing gingival inflammation, pocket formation and alveolar bone loss[(1)]. Though the etiology of periodontitis is multifactorial, but bacterial species are considered as the primary causative agents[(2)]. Periodontal tissue destruction is mediated either through direct action of bacteria on host tissues or by releasing various enzymes and endotoxins[(3)]. Cinical outcome of periodontal therapy are influenced by the presence or absence of particular microorganisms, notably Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans. Evidences indicate that scaling and root planing alone cannot predictably eliminate these tissue invasive micro organisms, thus it is proposed to boost synergistic effect of mechanical therapy with antimicrobial agents[(4)].

The efficacy of scaling and root planing (SRP) in etiotrophic phase is well evidenced in several longitudinal studies[(5)]. Hence periodontal therapy is necessarily directed at controlling the associated bacterial pathogens[(6)]. Chemotherapeutic agents have been used as adjuncts in the management of periodontal disease for many years and their role has recently been re-reviewed[(7)]. The repeated, long-term use of systemic Chemotherapeutic agents, is fraught with potential dangers, including resistant strains, superimposed infection and lack of patient compliance[(8)]. Additionally, systemic antibacterial agents have relatively low bioavailability in GCF even after high dosages of administration as compared to local drug delivery[(9)]. Advances in the technology of local drug delivery system have resulted in a number of site-specific, controlled- release methods to eliminate pathogenic microrganisms.

Several Chemotherapeutic agents (e.g. tetracyclines, metronidazole, chlorhexidine, clarithromycin and azithromycin) have been tested for LDD use in periodontal therapy[10]. Tetracyclines are broad-spectrum antibiotics with an instrumental role in reducing collagen and bone destruction by dual mechanism of action. Tetracyclines increase reattachment and regeneration by enhancing fibroblast activity and conditioning of root surfaces by impeding the collagenase activity as well as have a prominent bacteriostatic effect against periodontal virulent[11].

Hence this clinical study was conducted to compare the clinical and microbiological efficacy of collagen impregnated tetracycline fiber (Periocol-TC) as LDD when used as an adjunctive therapy to SRP in the treatment of chronic periodontitis. (Fig. 1)

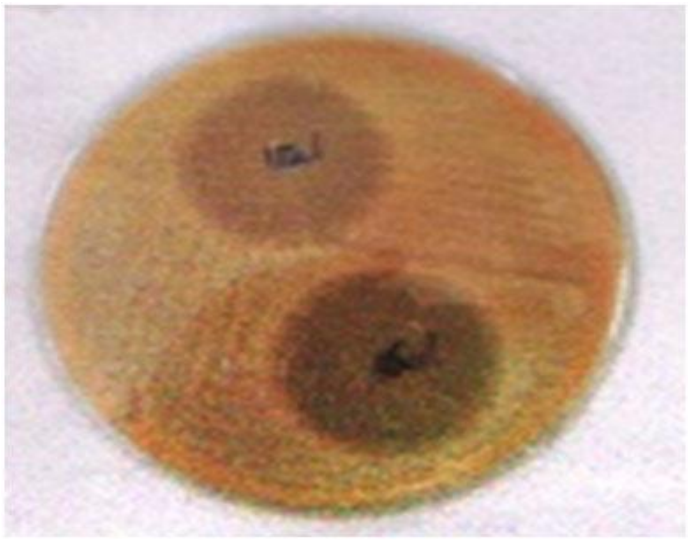


**Figure : 1** Periocol –TC drug for in vitro efficacy against Porphyromonas gingivalis

## II. Material And Methods

### 2.1 Source of data

This clinical study was conducted in the Department of Periodontics, Hitkarini Dental College and Hospital, India. The study design was approved by ethical committee of the college. Written informed consent form, explaining the nature of the study and procedure for local drug delivery was signed by all the patients.

### 2.2 Study design

Prospective randomised controlled trial with split mouth design.

### 2.2 Selection of patients

50 patients with 100 sites of chronic periodontitis including both males and females in the age group of 30– 55 years were selected. Patients with probing depth of ≥5 mm with radiographic evidence of bone loss and good oral hygiene, without any history of systemic disease were selected. Patients with poor oral hygiene and smokers were excluded from the study.

### 2.3 Clinical trial design

100 sites were randomly (by toss of a coin) divided into two groups of 50 each. An experimental group of 50 sites were designated as Group A and were treated with scaling, root planing and placement of Periocol-TC. Group B was a control Group of 50 sites treated by scaling and root planing alone. Plaque samples were collected (Fig. 2) at zero (baseline), $15^{th}$ and $45^{th}$ day and transported in thyoglycollate medium for quantitative and qualitative analysis of Porphyromonas gingivalis (Fig. 3). Meticulous scaling and root planing was performed for all the sites at zero (baseline) day. Additionally, Periocol-TC placement in the experimental sites was followed by dressing of periodontal pack, which isolated the area and restricted the effect of the tetracycline fibers to particular sites for at least a week. Patients of Group A were instructed, not to floss and probe the area with tongue, finger or toothpick. Additionally, patients were instructed to report immediately, if the material or pack was dislodged before the scheduled recall visit or if any pain, swelling or irritation occured.

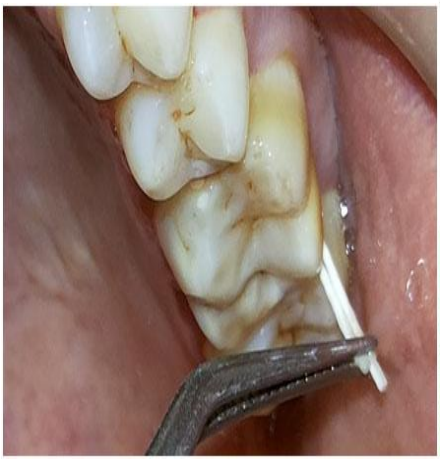


**Figure : 2** collection of plaque sample

**Figure :3** transport media ( thyoglycolate broth ) for P. gingivalis

Clinical parameters, including Plaque index (Silness and Loe, 1964), Gingival index (Loe and Silness, 1963), Pocket depth and Clinical attachment level was be recorded at 0 day (baseline), $15^{th}$ and $45^{th}$ day. A custom made acrylic stent and UNC-15 periodontal probe was used to standardize the measurement of pocket depth and relative attachment level. Samples were collected with complete aseptic precautions. Initially the site of sample collection was isolated with cotton rolls, carefully cleaned with sterile cotton pellets, and air-dried. For single sites, two sterile paper points were inserted to the bottom of the pocket for a 20-sec period and then transferred to thioglycollate medium.

**2.4 Periodontal status assessment**

***2.4.1 Probing pocket depth:*** The pocket depth was measured using a Hu- Freidy UNC 15 probe from fixed reference point (FRP) on the stent to the base of the pocket (BOP) and from the fixed reference point to the free gingival margin (GM). Probing pocket depth was obtained by subtracting distance of the Fixed Reference Point to Gingival Margin from Fixed Reference Point to Base of Pocket. The measurement was recorded to the nearest 1 mm and the recording was entered into the customized Performa made for the study for each patient. The customized occlusal stents were stored on the prepared study casts to minimize distortion and to record on the $45^{th}$ day.

***2.4.2 Clinical attachment level:*** The customized occlusal stent was placed on the selected teeth and the probe was gently inserted along the groove on the stent and the distance from the Fixed Reference Point (FRP) on the stent to the base of the pocket (BOP) and distance from Fixed Reference Point to the Cemento-Enamel Junction (CEJ) was recorded. Relative attachment level was obtained by subtracting the distance of Fixed Reference Point to Cemento Enamel Junction from Fixed Reference Point to Base of Pocket (Fig 4).

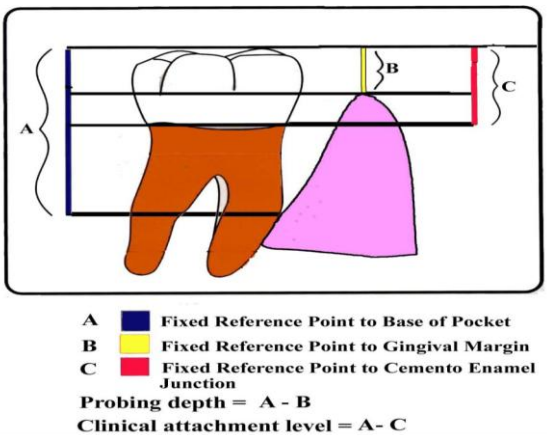



**Figure 4.** periodontal status assessment

***2.4.3 Tetracycline (Periocol –TC)***

Periocol- TC vial contains type I, fibrillar collagen of fish origin of approximately 25 mg, impregnated with approx. 2.0 mg of tetracycline hydrochloride IP, sterilized by gamma radiation with shelf life of 2 years. It releases tetracycline and it gets dissolve in the period of 8-12 days. It is manufactured by Eucare Pharmaceuticals, Chennai.

***2.4.4 In-vitro study***

**Assessment of the effect of Periocol –TC against Porphyromonas gingivalis in vitro study was conducted prior to clinical study and it was shown that Periocol –TC is effective against Porphyromonas gingivalis. (Fig 1)**

**Method:**

Porphyromonas gingivalis (ATCC 33277) was grown on blood agar medium. Using the agar-well diffusion method, two concentrations of the drug 15ul (approx. 3 collagen fibers in saline) and 25µl (approx. 3 collagen fibers in saline) were placed in the well. Upon incubation for 7 days, zone of inhibition around the wells were measured.

**Result:**

15 µl (approx. 3 collagen fibers in saline) showed zone of 21mm diameter. 25 µl (approx. 3 collagen fibers in saline) showed zone of 25mm diameter.

**Interpretation:** Periocol –TC is effective as locally delivered antibacterial agent against Porphyromonas gingivalis , (Fig no. 2)

***2.4.5 Collection of samples***

Samples were collected with complete aseptic precautions. Initially the site of sample collection was isolated with cotton rolls, carefully cleaned with sterile cotton pellets, and air- dried. For single sites, two sterile paper points were inserted to the bottom of the pocket (Fig no.2) for a 20-sec period and then transferred to thioglycollate medium (Fig no.3) and Periocol-TC, the tetracycline fibers were inserted in experimental sites. (Fig no.4).

***2.4.6 Microbiological examination***

The samples were processed within 24 hours. For isolation of strict anaerobes, the samples were plated on non-selective blood agar plates (5%) supplemented with hemin and menadione. Kanamycin-Vancomycin blood agar plates were used for selective growth of obligate anaerobic Gram-negative rods. The plates were incubated in vacuum desicator at 37ºC under anaerobic conditions for 7 days. After 7 days of incubation, colonies with differing characteristics were subjected to various tests. Identification was based on cell morphology, Gram stain reaction, biochemical and enzymatic tests including Indole test, Methyl red test, Voges- proskeur test, Citrate test, Carbohydrate fermentation test, Gas production test ( $H_2s$), Catalase test and Protease activity. (Figure no 5 & 6)


**Figure :3** biochemical test for confirmation of p.gingivalis

**2.4.6 Data analysis:** Independent't' and Paired 't' test, were employed in the present study for analysis of data using SPSS for Windows (Statistical Presentation System Software, 1999, SPSS Inc, New York) version 10.0.

## III. RESULTS

### 3.1 Plaque index

The mean plaque index scores of experimental and control groups at baseline were 2.335 ± 55082.0 (90%) and 2.3875 ± 67472.0(95%) which were reduced to 1.4125±0.27067 (39.50%) and 1.4500±0.27625 (38.90%) at 45$^{th}$ day respectively. On comparing between the two groups no significant differences were observed. **(TABLE-1, GRAPH 1)**

**Table :1** Mean Standard Deviation Of Plaque Index Before And After Treatment

| Experimental group | | | Control group | | |
|---|---|---|---|---|---|
| | **PI** | **Change from baseline** | **PI** | **Change from baseline** | **P. value** |
| **Baseline** | 2.335 ± 55082.0 | | 2.3875± 67472.0 | | t=0.5979;p>0.05 |
| **At15$^{th}$day** | 0.5525±0.31808 | 75.7% | 0.5750±0.34124 | 39.5% | t=0.2157;p>0.05 |
| **At45$^{th}$day** | 1.4125±0.27067 | 75.8% | 1.4500±0.27625 | 38.9% | t=0.4336;p>0.05 |

### *3.2 Gingival index*

The mean gingival index scores of experimental and control groups were 1.17 ±0.568 and 1.35 ±0.489 which were reduced to 1.025 ±0.4128 (25.6 %) and 1.055 ±0.4419 (28.0 % ) at 45$^{th}$ day respectively. On comparing between the two groups no significant differences were observed. **(**TABLE-2, GRAPH 1)

**Table :2** Mean Standard Deviation Of Gingival Index Before And After Treatment

| Experimental group | | | Control group | | |
|---|---|---|---|---|---|
| | **GI** | **Change from baseline** | **GI** | **Change from baseline** | **P. value** |
| **Baseline** | 1.17±0.568 | | 1.35±0.489 | | t=1.0435;p>0.05 |
| **At15$^{th}$day** | 0.74±0.681 | 53.1% | 0.56±0.399 | 25.6% | t=0.9917;p>0.05 |
| **At45$^{th}$day** | 1.025±0.4128 | 62.5% | 1.055±0.4419 | 32.3% | t=0.2219;p>0.05 |

### 3.3 Probing pocket depth

The mean reduction in probing pocket depth of experimental and control groups at baseline were 5.8 ±1.105 and 6.1 ±1.252 which were reduced to 3.9 ±1.119 (33.7%) and 4.25 ±1.251(31.0%) at 45$^{th}$ day respectively. On comparing between the two groups no significant differences were observed. **(TABLE-3,** GRAPH 1**)**

**Table :3** Mean Standard Deviation Of Probing Pocket Depth Before And After Treatment

| Experimental group | | | Control group | | |
|---|---|---|---|---|---|
| | **PPD** | **Change from baseline** | **PPD** | **Change from baseline** | **P. value** |
| **Baseline** | 5.8±1.105 | | 6.1±1.252 | | t=0.8033;p>0.05 |
| **At45$^{th}$day** | 3.9±1.119 | 33.7% | 4.25±1.251 | 31.0% | t=0.9324;p>0.05 |

### 3.4 Clinical attachment loss

The mean clinical attachment loss of experimental and control groups from baseline was 13.15 ±1.631 and 14.45 ±2.481, which was reduced to 11.8 ±1.473 (10.1%) and 12.95 ±2.523 (10.6% ) at the end of 45$^{th}$ day respectively. On comparing the two groups no stastistically significant differences was observed. **(TABLE-4. &** GRAPH 1)

**Table :4** Mean Standard Deviation Of Clinical Attachment Level Before And After Treatment

| Experimental group | | | Control group | | |
|---|---|---|---|---|---|
| | **CAL** | **Change from baseline** | **CAL** | **Change from baseline** | **P. value** |
| **Baseline** | 13.15±1.631 | | 14.45±2.481 | | t=1.9581;p>0.05 |
| **At45$^{th}$day** | 11.8±1.473 | 10.1% | 12.95±2.523 | 10.6% | t=1.7605t=p>0.05 |

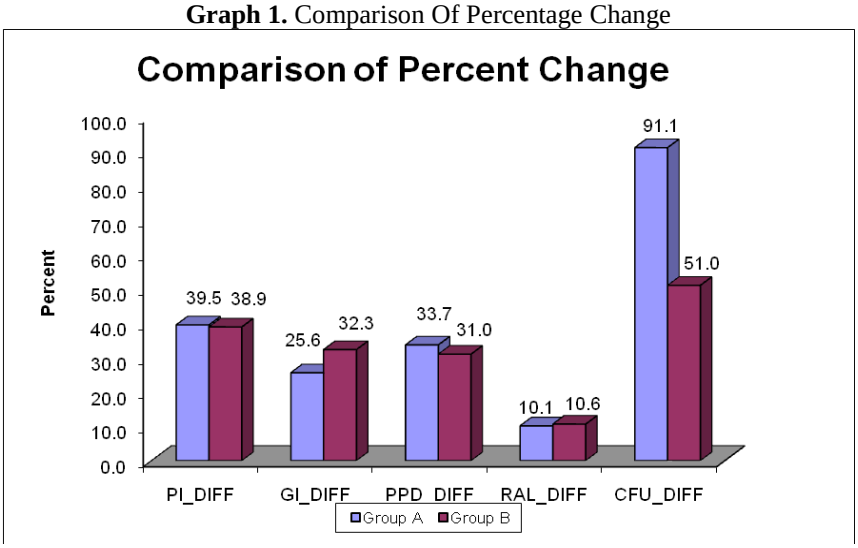
### 3.5 Colony forming units

The mean colony forming units / ml in experimental and control groups at baseline was 245.5 ±63.071 and 289.45 ±55.618 which were reduced to 76.35 ±26.900 (65.9%) and 76.25 ±21.108 (73.6%) at 15$^{th}$ day and 18.57±9.748 (91.1%) and 136 ±26.983 (51.0%) at 45$^{th}$ day. On comparison between the two groups experimental group revealed
(91.1%) reduction in colonies of P.gingivalis compared to (51.0%) control group. Although there was an increase in colonies of P.gingivalis from 15$^{th}$ to 45$^{th}$ day in control group. **(TABLE-5 &** GRAPH 1)

**Table :5** Mean Standard Deviation Of Colony Forming Units Before And After Treatment

| Experimental group | | | Control group | | |
|---|---|---|---|---|---|
| | **CFU** | **Change from baseline** | **CFU** | **Change from baseline** | **P. value** |
| **Baseline** | 245.5±63.071 | | 289.45±55.618 | | t=2.3374;p<0.05 |
| **At15$^{th}$day** | 76.35±26.900 | 65.9% | 76.25±21.108 | 73.6% | t=0.0131;p>0.05-0 |
| **At45$^{th}$day** | 18.57±9.748 | 91.1% | 136±26.983 | 51.0% | t=17.8676;p<0.0001 |

**Graph 1.** Comparison Of Percentage Change




## IV. Discussion

Periodontal management principally attempts to slow disease progression, prevent re-occurrence of disease and re-generate or repair tissues that have been lost or damaged. Although scaling and root planing reduces the number of periodontal pathogens, however complete eradication of invasive micro-organisms from the underlying connective tissue is un achievable by non surgical therapy alone[(3)].

Adjunctive use of chemotherapeutic agents either in the form of systemic or local delivery systems is designed to overcome the in-efficiency of the conventional treatment[(10)]. Systemically administered chemotherapeutic agents achieve relatively low concentrations in pockets, even after high dosages. Local applications of chemotherapeutic in direct contact with the root surface can reduce or eliminate pathogenic organisms that could not be eradicated mechanically[(6)].A local route of drug delivery can attain 100-fold higher concentration of an antimicrobial agent in subgingival sites compared with a systemic drug regimen[(12)].

Tetracycline is an antibiotic isolated from *St. aureofaciens*. Chemically, it is the hydrochloride of 4 - dimethylamino - 1, 4, 4a,5,5a,6,11,12a-octahydro- 3,6,10,12,12a- pentahydroxy-6- methyl – 1, 11 - di oxo - 2-

napthacene carboxamide. Tetracycline hydrochloride and doxycycline, are broad- spectrum antibiotics that are effective against obligate and facultative anaerobes. Tetracyclines are bacteriostatic for many pathogens at concentrations found in the gingival crevicular fluid after systemic administration (3-6 microgram/ml). However, local delivery of these drugs provides high concentrations that are bacteriocidal. Local application of tetracycline has been associated with minimal side effects including difficulty in placing therapeutic concentrations of the antimicrobial agent into deeper parts of periodontal pockets and furcation lesions. Personal application of antimicrobial agents by patients as a part of their home selfcare procedures is frequently compromised by the patient's lack of adequate manual dexterity, limited understanding of periodontal anatomy and poor compliance[12].

Various commercially available tetracycline products used as local drug delivery are Actisite, 2% minocyclin as dentomycin, Periodontal plus AB, Periocol –TC. Periocol-TC is commercially available as a vial of 2.0 mg; It contains type I, fibrillar collagen of fish origin, approximately 25 µg, impregnated with approx. 2.0 µg of tetracycline hydrochloride IP, sterilized by gamma radiation with shelf life of 2 years. It releases tetracycline by getting dissolved within the period of 8-12 days[5].

In fish, the largest concentration of collagen is found in the skeleton, fins, skin and air bladder. The role of collagen as a vehicle for drug delivery is a well documented subject (Collagen is an extra cellular matrix protein playing a major role in connective tissue.It is the most abundantprotein in human and performs multiple functions. It is biocompatible and absolutely safe for human applications. PerioCol®-TC must be stored in a dry place at between 5° C and 25° C. It is gamma sterilized with a shelf life of 2 years. Manufactured by Eucare pharmaceuticals pvt LTD Chennai[6].

The percentage improvement in mean gingival index score in experimental and control groups from baseline to 15$^{th}$ day was 62.5% and 53.1%, respectively. While comparing from baseline to 45$^{th}$ day, a mean change of 0.15 was observed with a't' value of 1.18 (25.6%) and 0.29 with a't' value of 2.25 (32.3%). Thus no significant difference between the two groups at 15$^{th}$ day and 45$^{th}$ day

There was no statistically significant difference found in both probing pocket depth and clinical attachment level (CAL) in both experimental and control groups at the end of 45th day. The **mean pocket depth reduction** for experimental and control group from baseline to 45$^{th}$ day was 1.900 with't' value of t=27.606 (33.7%) and 0.587 with't' value of t=14.091 (31.0%) respectively and mean clinical attachment level for experimental and control group from baseline to 45$^{th}$ day was 1.350 with't' value of t=7.429 (10.1%) and 0.688 with 't' value of t=9.747 (10.6%).

Similar findings were observed in the studies conducted by Newman MG et al, 1994[14] and Chandrashekar KT and Sinha S, 2011[15].

Microbial parameters were assessed by collection of plaque sample on 0 (baseline), 15$^{th}$ and 45$^{th}$ day for quantitative and qualitative analysis of Porphyromonas gingivalis, On comparing the colony forming units per ml from baseline to 15$^{th}$ day, mean change of 169.150 with 't' value of t= 10.404 indicates reduction up to 65.9% and 213.200 with 't' value of t= 22.323 showed reduction up to 73.6% in experimental and control groups respectively, indicating no statistical difference among two groups. Whereas on comparing from baseline to 45$^{th}$ day, mean change of 223.286 with't' value of t= 13.228 showed reduction up to 91.1% and 55.831 with a't' value of t= 12.292 showed reduction up to 51.0% in experimental and control groups respectively. Thus it signifies that Periocol –TC is able to reduce the level of Porphyromonas gingivalis, in experimental group where as control group shows increase in colonies of Porphyromonas gingivalis. Similar findings were observed in the studies conducted by Mombelli et al in 1996[16].

## V. Conclusion

This study was aimed on evaluating clinical and the microbiological efficacy of (tetracycline fibers) Periocol – TC in the treatment of patients with Periodontitis. From the results of this study it can be summarized that: there was a statistically significant reduction in PD with gain in CAL of groups from baseline and 45$^{th}$ day and control. However, on comparison between the two groups no significant difference was found.

There was a statistically significant reduction in the colonies of Porphyromonas gingivalis, in control group (73.6%) and experimental group (65.9%) from baseline to 15$^{th}$ day, however on comparing the groups from 15$^{th}$ to 45$^{th}$ day experimental group showed 91.1% reduction in level of Porphyromonas gingivalis, which is highly significant. Whereas in control group showed recolonization of Porphyromonas gingivalis was observed.

According to this clinical study, the controlled, noninvasive, local drug delivery system using tetracycline fiber (Periocol-TC) turned out to be a simple and rapid procedure. It appears to be a useful treatment modality to reduce the level of Porphyromonas gingivalis, as well as offers more favorable clinical attachment gains and probing pocket depth reductions for patients with chronic periodontitis.

## Acknowledgement

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