Original Article

Evaluation of clinical and antimicrobial efficacy of chitosan nanoparticle gel (1%) as a local drug delivery in chronic periodontitis patients- a randomised controlled split-mouth trial

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ABSTRACT

Background

Chitosan is a naturally derived polymer that has been extensively investigated for its use as a biomaterial for local drug delivery and anti-inflammatory activity. Recently, chitosan applications in periodontal tissue healing have gained significant interest.

Objectives

The present study was undertaken to evaluate the efficacy of chitosan nanoparticle gel when used as an adjunct to scaling and root planing in terms of clinical outcomes and antimicrobial activity.

Methods

The study was conducted following a split-mouth design in which patients with chronic periodontitis were selected and test and control sites were randomized. Test sites received chitosan nanoparticle gel administration adjunct to scaling and root planning while control sites were subjected to scaling and root planing. Subgingival plaque samples were collected for RT-PCR analysis at baseline and follow-up after 6 weeks.

Results

It was observed that there was a significant reduction in PI (p-value: 0.002) and PD scores (p-value: 0.001) along with a gain in CAL (p-value: 0.001) in the test group when chitosan nanoparticle gel (1%) was used as an adjunct to SRP. There was no statistically significant difference seen with mean GI scores (p-value: 0.387). Further, chitosan nanoparticle administration significantly reduced counts of P.gingivalis and T.forsythia in the test sites than the control sites.

Conclusion

Administration of 1% chitosan nanoparticle gel adjunct to scaling and root planning in chronic periodontitis patients resulted in a significant improvement in clinical parameters while exhibiting inhibitory action against the periodontal pathogens P.ginigivalis and T.forsythia.

Keywords

Chitosan; nanoparticles; periodontitis; local drug delivery; microbial analysis; semiquantitative PCR

INTRODUCTION

An infectious illness known as chronic periodontitis is characterised by elevated inflammation in the tissues that support the teeth, which eventually results in increasing attachment loss and bone loss. [1] A significant rise in the number of bacteria colonising the pockets is the cause of the disease's occurrence and progression, and host- and environment-related factors further alter this number.

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When compared to other therapeutic treatments, scaling and root planning (SRP), the first stage in treating periodontal disorders, has been considered the "gold" standard therapy for both the initial and maintenance phases of the condition. [2] Reduced probing pocket depth and increased attachment level are two of the clinical outcomes of SRP, which balance out most cases of periodontitis. Nevertheless, mechanical therapy is insufficient to completely remove the bacteria from the pockets when tissue-invading pathogens like Actinobacillus actinomycetemcomitans (Aa) or Porphyromonas gingivalis (Pg) are present. Thus, rapid progression of attachment loss and alveolar bone resorption occurs despite comprehensive treatment. Accessory antibacterial therapy offers a benefit in these situations. [3,4,5,]

Systemic antimicrobials are helpful, but they have drawbacks as well. For example, the drug can cause antibiotic resistance when taken in large doses, and hepatic bypass lowers the drug's concentration in GCF.[6] Additionally, systemic antimicrobials have side effects that make patients less tolerant of the medication. As a result, alternate methods of medication delivery are required, such as the direct application of controlled release delivery agents to the location of need.

According to studies found in the literature, local drug delivery after scaling and root planing has been shown to have positive outcomes in locations that are nonresponsive to conventional therapy [7]. However, it should be used as a supplement, not a replacement for conventional therapy. [8] The ultimate goal is to guarantee the drug's continuous availability in adequate minimum inhibitory concentrations over a crucial period of time in order to eradicate any remaining infectious or inflammatory component that is still shielding the periodontal tissues that are inaccessible by hand or with motorised instruments. [7]

Many locally accessible drug delivery agents are commercially available; these include Actisite (tetracycline), Atridox (doxycycline), Arestin (minocycline), Elyzol (metronidazole), and Periochip (chlorhexidine). While these agents are beneficial, they still have certain limitations, such as resistance and substantivity 9, 10, As a result, there is constant research into novel, reasonable, and naturally occurring medicinal substances. Alkaline deacetylation of chitin results in the production of chitosan, a linear chain polymer. It is made comprised of the positively charged amino group (NH3+), which damages bacterial cell walls and causes cell contents to leak when it interacts with the electronegatively charged surface of bacterial cells. Chitosan and its derivatives are used extensively in the medical field because of their excellent biocompatibility, biodegradability, selective permeability, mucoadhesiveness, antimicrobial activity, anti-inflammatory, bone repair, and wound healing properties. They can also be processed into a variety of forms, including blends, solutions, sponges, membranes, gels, pastes, tablets, microspheres, and microgranules. [11]

Studies on the effectiveness of chitosan in treating patients with chronic periodontitis, its effectiveness when applied as a gel, animal studies (like bone formation in rat calvarial defects), and in vitro studies (like MIC against period pathogens and anti-inflammatory activity in gingival fibroblasts) have all been published in the literature. To the best of our knowledge, there are no published studies on the antibacterial activity of chitosan nanoparticle gel or its usage as a local drug delivery system for the treatment of chronic periodontitis.Studies on the effectiveness of chitosan in treating patients with chronic periodontitis, its effectiveness when applied as a gel, animal studies (like bone formation in rat calvarial defects), and in vitro studies (like MIC against period pathogens and anti-inflammatory activity in gingival fibroblasts) have all been published in the literature. To the best of our knowledge, there are no published studies on the antibacterial activity of chitosan nanoparticle gel or its usage as a local drug delivery system for the treatment of chronic periodontitis. Hence the aim of this study was i) to evaluate the clinical efficacy of Chitosan nanoparticle gel (1%) as local drug delivery in patients with chronic periodontitis ii) to evaluate the antimicrobial efficacy of Chitosan nanoparticle gel (1%) as local drug delivery against periodontal pathogens namely Porphyromonasgingivalis and Tannerella forsythia and iii) to compare the efficacy of Chitosan nanoparticle gel (1%) with SRP in the treatment of chronic periodontitis. The study hypothesis was that adjunctive use of Chitosan nanoparticle gel (1%) to scaling and root planing exhibited antimicrobial activity against periopathogens:-P.gingivalis, T.Forsythia.

MATERIALS AND METHODS

The study was designed as a randomized controlled split-mouth clinical trial; ethical principles of the

Declaration of Helsinki (2002) were followed and approved by the Institutional Ethical Committee. The study was conducted in the Department of Periodontics, Dayananda Sagar College of Dental Sciences, Bengaluru with an enrollment of 26 patients in the age group of 20-60 years after fulfilling the selection criteria. A written informed consent was obtained from all the participants.

RANDOMISATION

Test and control sites were determined by the coin toss method.

ELIGIBILITY CRITERIA FOR PARTICIPANTS

Patients in the age group 20-60 years diagnosed with chronic periodontitis having probing depths of 5-8mm and clinical attachment level of 3mm were included in the study while those with a history of any systemic diseases, periodontal treatment in the last 6 months, pregnant and lactating women, smokers, alcoholics, and drug abusers were excluded from the study.

The study was conducted in a split-mouth way contralateral sites with probing pocket depth 5-8 mm in similar teeth (premolars and molars) in the maxillary arch were selected. Periodontal parameters like Plaque index (Silness and Loe), gingival index (Loe and Silness), probing pocket depth measurement and clinical attachment loss were recorded using a UNC 15 periodontal probe. (Figure.1) The area was isolated, supragingival plaque was removed, sub gingival plaque samples were collected from both test and control sites (**figure 2 A**). The samples were immediately transferred to Eppendorf tubes containing tris EDTA buffer. Complete scaling was done followed by root planing at the test and control site using area-specific curettes (Hu-Friedy 9/10, 15/16, 17/18). After isolation of the test site using sterile cotton rolls Chitosan nanoparticle gel was administered using a syringe with a blunt cannula till the entire pocket was loaded with the drug **(figure 2 B).** The pocket entrance was covered with the periodontal pack to retain the material in the pocket **(figure 2 C).** The vials containing the samples were appropriately labelled and stored at -80^0 C. Subjects were called back after 7 days for removal of the periodontal dressing. During this time, they were instructed to refrain from brushing and flossing the area and were instructed not to disturb the area with their tongue/finger. They were further instructed not to use any chemical plaque control agents. The subjects were recalled after 6 weeks for assessing the clinical parameters followed by plaque sample collection.

PCR Procedure:

Agar gel electrophoresis was used for the identification and quantification of bacteria namely, P. gingivalis and T. forsythia (**figure 3**). Figure 4 shows the preoperative and postoperative conditions. Microbial analysis was done by semi-quantitative PCR (**Figure 5**)

Study outcomes:

The outcomes of the study were 1) clinical parameters namely plaque index, gingival index, probing depths and clinical attachment levels ii) microbial counts of P. gingivalis and T. forsythia.

Sample size determination:

Using G power software version 3.1.9.2, with a power of 0.80, α-error of 0.05 and effect size of 0.5, a sample size of 26 was obtained.

Statistical analysis:

Normality tests Kolmogorov-Smirnov and Shapiro-Wilks tests results revealed that variables do not follow Normal distribution. Therefore, to analyze the data, the non-parametric method was applied. The study design was a "split mouth design" and hence to compare values between groups and between time points Wilcoxon Signed Rank test was applied. Spearman's Rank correlations were estimated to assess the linear relationship between clinical and microbiological parameters. In the above tests, a P value less than 0.05 was considered to be statistically significant. For analysis of the data, SPSS Version 26.0 was used.

Ethical clearances: Ethical clearance has been obtained from the Institutional review board and an ethical certificate has been attached

RESULTS

A total of 26 patients were enrolled in the study. 2 patients refused to turn up for follow up and hence remaining 24 were followed up. Demographic data of participants are shown in **Table 1**

Intragroup analysis after 6 weeks revealed statistically significant results in the test group concerning PI (p-value:0.002), PD (p-value: 0.001) and CAL (p-value: 0.001) than in the control group where PI (p-value: 0.04), PD (p-value: 0.01) and CAL (p-value: 0.04) **(tables 2&3). (Figure.7,8,9)**

Intergroup analysis after 6 weeks revealed that there was a statistically significant reduction seen with mean PI (p-value: 0.05) and mean GI (p-value: 0.03) in the test group compared to mean PI (p-value: 0.887) and mean GI (p-value: 0.460) in the control group. However, mean PD (p- 0.6) and mean CAL (p- 0.3) in the test sites at 6 weeks were not statistically significant compared to mean PD (p-value:0.06) and mean CAL (p-value:0.06) in the control sites **(tables 4&5).**

Intragroup analysis revealed that in the control group, there were no significant reductions in Pg counts (p-value: 0.563) but a significant reduction was seen in Tf (p-value: 0.094) while in the test group, there was a significant reduction in counts of both Pg (p-value: 0.023) and Tf (p-value: 0.007)**.**

Comparison of microbial counts between the test and control sites at baseline did not show any significant reduction in Pg (p-value: 0.600) and Tf (p-value: 0.819). However, at the end of 6 weeks, mean Pg counts expressed a statistically significant reduction (p-value: 0.007) but no significant reduction was seen with Tf counts (p-value: 0.135)**.**

Correlation tests revealed that in control sites the mean PI scores expressed mild correlation with Pg at baseline (R-value:-0.239) as well as at 6 weeks (R-value:-0.267) and also with Tf at baseline (R-value:-0.257) and at 6 weeks (R- value:-0.211) while in test sites there was no correlation with counts of Pg at baseline (R-value: 0.034) and mild correlation at 6 weeks (R-value: -0.127). However, no correlation was observed with Tf at baseline (R-value: 0.057) as well as after 6 weeks (R-value: 0.58).

In control sites, mean GI scores expressed moderate correlation with Pg at baseline (R-value:-0.313) as well as at 6 weeks ((R-value:-0.462). Also, moderate correlation was observed with Tf at baseline and no correlation at 6 weeks (R-value:-0.45) while in test sites, mean GI scores expressed mild correlation with Pg at baseline (R-value: 0.151) and at 6 weeks (R-value:1.72). A moderate correlation was observed with Tf at baseline (0.383) and no correlation at 6 weeks (R-value:0.89).

In control sites, at baseline, mean PD scores exhibited mild correlation with Pg (R-value:0.280) and moderate correlation with Tf (R-value:0.399) while at 6 weeks, mean PD scores exhibited no correlation with Pg (R-value: 0.050) and mild correlation with Tf (R-value: 0.295). In test sites mean PD scores showed a mild correlation with Pg (R-value: 0.122) and no correlation with Tf (R-value: 0.005). At 6 weeks also, mean PD scores showed a mild correlation with Pg (R-value:0.159) and no correlation with Tf (R-value:-0.034). (Figure 10)

In control sites, a moderate correlation of CAL was observed with Pg (R-value:0.313) and a strong correlation with Tf (R-value:0.504) (table 2)while at 6 weeks, no correlation was observed with Pg (R-value:0.81) and Tf (R-value: 0.304)(table 3).In test sites mean CAL scores showed mild correlation with Pg (R-value: 0.168) and no correlation with Tf (R-value: 0.001) (table 4).Mean CAL scores showed no correlation with Pg (R-value:0.016) and mild correlation with Tf (R-value:0.170) (Figure.11)

DISCUSSION

Gram-negative bacteria and the host immune system combine to cause periodontitis, a chronic inflammatory disease. [12,13,14] This condition is linked to the development of periodontal pockets, clinical attachment loss, and radiographic evidence of bone loss. Genetic, systemic, environmental, and other variables might exacerbate these effects. In reaction to the invasive bacteria, the host mounts an immunological and inflammatory defence by releasing a range of inflammatory mediators that lead to the degeneration of bone and connective tissue. [15] Because these organisms are tissue invasive, such as Porphyromonas gingivalis and Actinomyces actinomycetemcomitans, mechanical therapy is insufficient to remove them. Thus, adjuvant antibacterial therapy is required. In this work, the effectiveness of chitosan nanoparticle gel as a supportive antibacterial agent was assessed. After the administration of chitosan gel, there were no allergic or inflammatory reactions. Chitosan-containing formulations are anticipated to remain on the application site for an extended period of time due to their bio-adhesive characteristics. [16] To the best of our knowledge, this is the first study of its kind to limit the effectiveness of a local drug delivery system using 1% chitosan nanoparticle gel against P. gingivalis and T. forsythia in vivo.

It was observed in our study, on intragroup comparison, there were statistically significant reductions observed with PI (p-value: 0.048), PD (p-value: 0.012) and gain in CAL (p-value: 0.046) in control sites as well as PI (p-value: 0.002), CI (p-value:0.001) and CAL (p-value:0.001) in test sites from baseline to 6 weeks.

On intergroup comparison between control and test sites, there were statistically significant reductions in mean plaque index (p-value: 0.051) and mean gingival index scores (p-value: 0.003).

These outcomes were similar to studies done by Bayati et al where the efficacy of chitosan chips in chronic periodontitis patients was evaluated and Akncbay et al where chitosan incorporated with metronidazole gel and chitosan (1%) was evaluated. [17]

Substantial improvements in terms of clinical outcomes namely PI, GI, BOP, PD and CAL were reported by Babrawala et al and Kodega et al in response to the use of chitosan membrane and simvastatin and chitosan when used as an adjunct to SRP [18,19] Gayasuddin et al reported favourable In our study, statistically significant decreases in PI (p-value: 0.048), PD (p-value: 0.012), and gains in CAL (p-value: 0.046) were seen in the control locations, while PI (p-value: 0.002), CI (p-value: 0.001), and CAL (p-value: 0.001) were seen in the test sites from baseline to six weeks. The mean gingival index and mean plaque index scores showed statistically significant decreases on intergroup comparison between control and test sites (p-value: 0.051). (p-value: 0.003).

These results were in line with research conducted by Bayati et al. to assess the effectiveness of chitosan chips in patients with chronic periodontitis and by Akncbay et al. to assess the efficacy of chitosan combined with metronidazole gel and chitosan (1 percent). [17] results in terms of clinical parameters while metronidazole and levofloxacin-loaded chitosan films are used as compared to metronidazole and levofloxacin alone. [20]

The present study also observed that there was a significant reduction seen in P. gingivalis (p-value: 0.023), as well as T forsythia (p-value: 0.007), counts in the test compared to control sites. An in vitro study done by Costa et al (2014) reported that 1% of chitosan had inhibitory action against P.gingivalis, P.intermedia, P buccal, T. forsythia and A a contains by preventing biofilm formation. Another in vitro study by Ikinsi et al (2002) displayed that chitosan when added to chlorhexidine resulted in superior inhibition of P gingivalis growth than chlorhexidine alone exhibiting the antimicrobial effect of chitosan. The study reported that chitosan particles showed antimicrobial activity against P gingivalis and A.acomitans [20,21,22,23] Other analogous studies regarding the significant The current study also found that, when compared to control locations, there was a substantial decrease in the counts of T forsythia (p-value: 0.007) and P. gingivalis (p-value: 0.023). According to an in vitro study by Costa et al. (2014), by inhibiting the production of biofilms, 1% of chitosan exhibited inhibitory effect against P. gingivalis, P. intermedia, P buccal, T. forsythia, and A a contains. Ikinsi et al. (2002) conducted another in vitro investigation that demonstrated the antibacterial action of chitosan by showing that adding it to chlorhexidine enhanced the inhibition of P gingivalis growth compared to using chlorhexidine alone. According to the study, chitosan particles exhibited antibacterial activity against A. acomitans and P gingivalis. [20,21,22,23] clinical improvement of gingival health could not be collaborated with other studies as the detailed perusal of the available literature failed to show any such similar study. As a result, it was impossible to compare our findings about the improvement in both groups with those of other authors. Therefore, the administration of 1% chitosan nanoparticle gel produced noticeably superior results after a 6-week break, supporting the medication's anti-inflammatory efficacy.

In a follow-up investigation, we attempted to assess radiographically the effectiveness of 1% chitosan nanoparticles in promoting the creation of new bone in infra-bony defects after 24 weeks. However, the unavoidable COVID-19 epidemic prevented us from finishing the analysis, which explains the study's limitations. Furthermore, since periodontitis is a complex illness linked to numerous pathogens, it is necessary to assess the effectiveness of chitosan nanoparticle gel against each and every one of these pathogens.

In order to better understand the role of chitosan nanoparticle gel among patients with stage III and chronic periodontitis, it is advised that additional research be done on the effectiveness of chitosan nanoparticle gel (1 percent) on other periodontal pathogens as well as in evaluating the radiographic bone fill in periodontal regeneration.

CONCLUSION

The results of our trial demonstrated that 1% chitosan nanoparticle gel stimulated a significant improvement in clinical as well as microbiological parameters. Hence it can be proposed that the use of chitosan gel (1%) when used as adjunct to scaling and root planing stimulates significant pocket depth reduction, and gain in clinical attachment levels thereby enhancing periodontal tissue

healing. This can provide a new direction in providing appropriate treatment for periodontal diseases. However, further long-term multicentre randomized, controlled clinical trials using different vehicles and concentrations of chitosan should be carried out to ascertain the clinical and microbiological effects of the drug.

Registration:

The trial is registered in Clinical Trials Registry India (CTRI) under the registration number CTRI/2019/05/019477.

Conflict of Interest: Authors declare there is no conflict of interest

Authors's contribution:

Data gathering and idea owner of this study: Dr.Savita A M

Study design: Dr. Pallavi Nanaiah, Dr Anupama Aradya

Data gathering: Dr Archana R Naik, Dr Nageshwaran G

Writing and submitting a manuscript: Dr.Koduru Sravani, Dr Anupama Aradya

Editing and approval of final draft: Dr.Koduru Sravani, Dr. Savita A M, Dr Anupama Aradya

TABLE 1: spearman's rank correlations between clinical parameters and microbiological parameters for control site (baseline)

		Reduction in PG - Control	Reduction in TF - Control
Mean PI-BL: Control	R-value	-.239	-.257
	P-value	.261	.226
Mean GI-BL: Control	R-value	-.313	-.328
	P-value	.137	.118
Mean PD-BL: Control	R-value	.280	.399
	p-value	.186	.053
Mean CAL-BL: Control	R-value	.313	.504

TABLE 2: spearman's rank correlations between clinical parameters and reduction in microbiological parameters for test site (baseline)

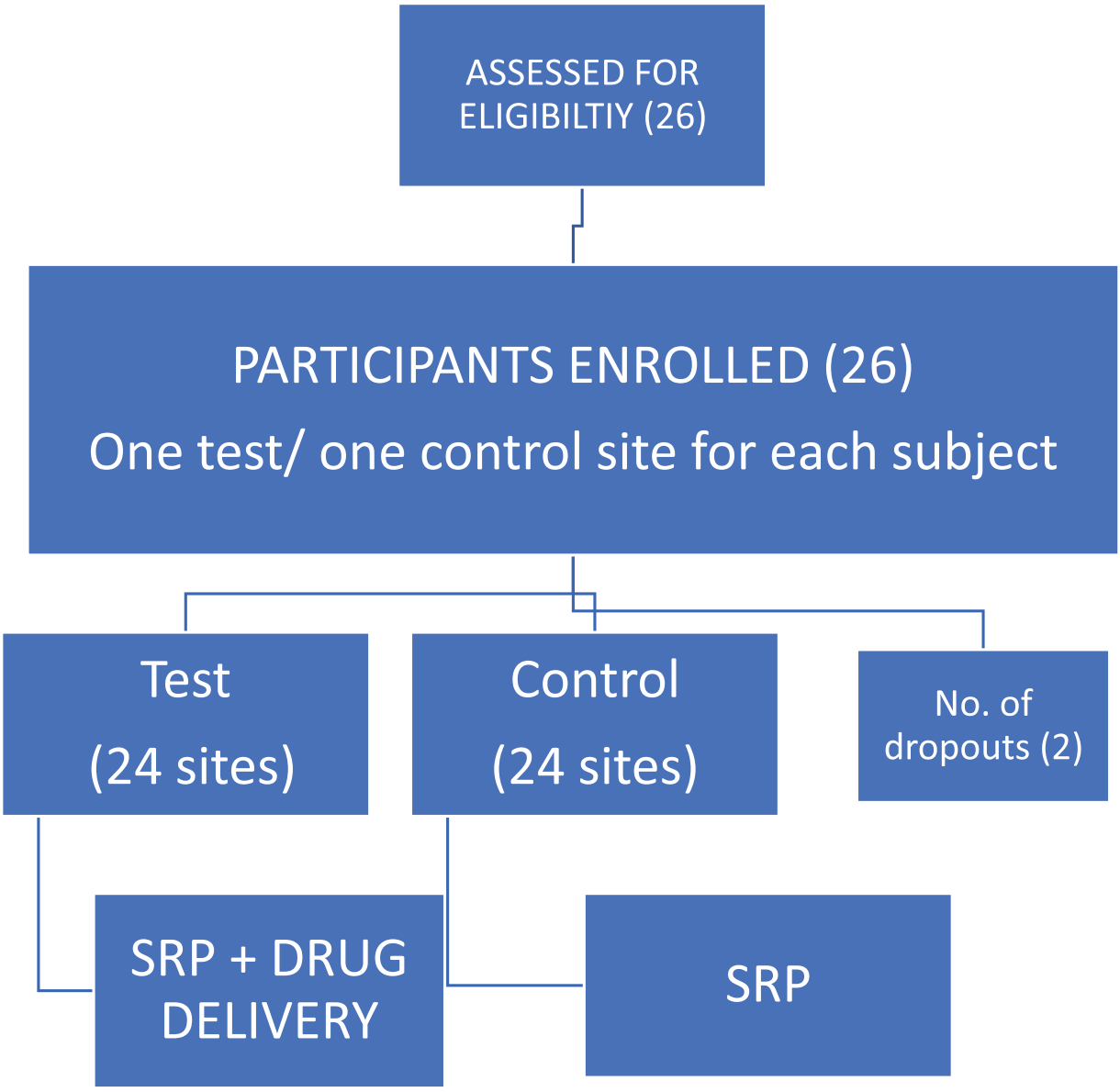
		Reduction in PG-Test	Reduction in TF - Test
Mean PI-BL: Test	R-value	.034	.057
	P-value	.874	.791
Mean GI-BL: Test	R-value	.151	-.383
	P-value	.480	.065
Mean PD-BL: Test	R-value	.122	-.005
	p-value	.570	.982
Mean CAL-BL: Test	R-value	.168	.001
	p-value	.434	.995

TABLE 3: spearman's rank correlations between clinical parameters and reduction in microbiological parameters for control site (at 6 weeks)

		Reduction in PG - Control	Reduction in TF - Control
Mean PI-6W: Control	R-value	-.267	-.211
	P-value	.207	.322
Mean GI-6W:Control	R-value	-.462	-.045
	P-value	.023	.834
Mean PD-6W: Control	R-value	.050	.295
	p-value	.817	.162
Mean CAL-6W: Control	R-value	.081	.304
	p-value	.708	.149

TABLE 4: spearman's rank correlations between clinical parameters and reduction in microbiological parameters for test site (6 weeks)

		Reduction in PG-Test	Reduction in TF - Test
Mean PI-6W: Test	R-value	-.127	.058
	P-value	.554	.787
Mean GI-6W:Test	R-value	.172	.089
	P-value	.422	.678
Mean PD-6W: Test	R-value	.159	-.034
	p-value	.459	.875
Mean CAL-6W: Test	R-value	.016	.170
	p-value	.942	.427



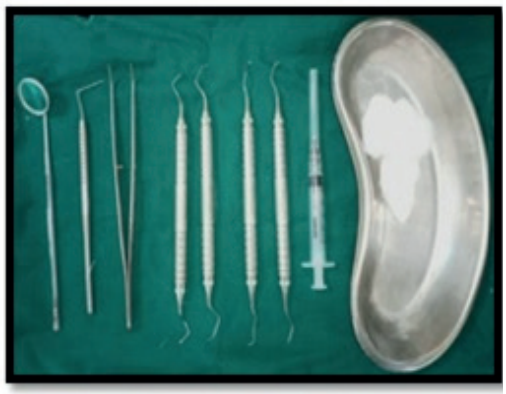


Figure: 1 Armamentarium

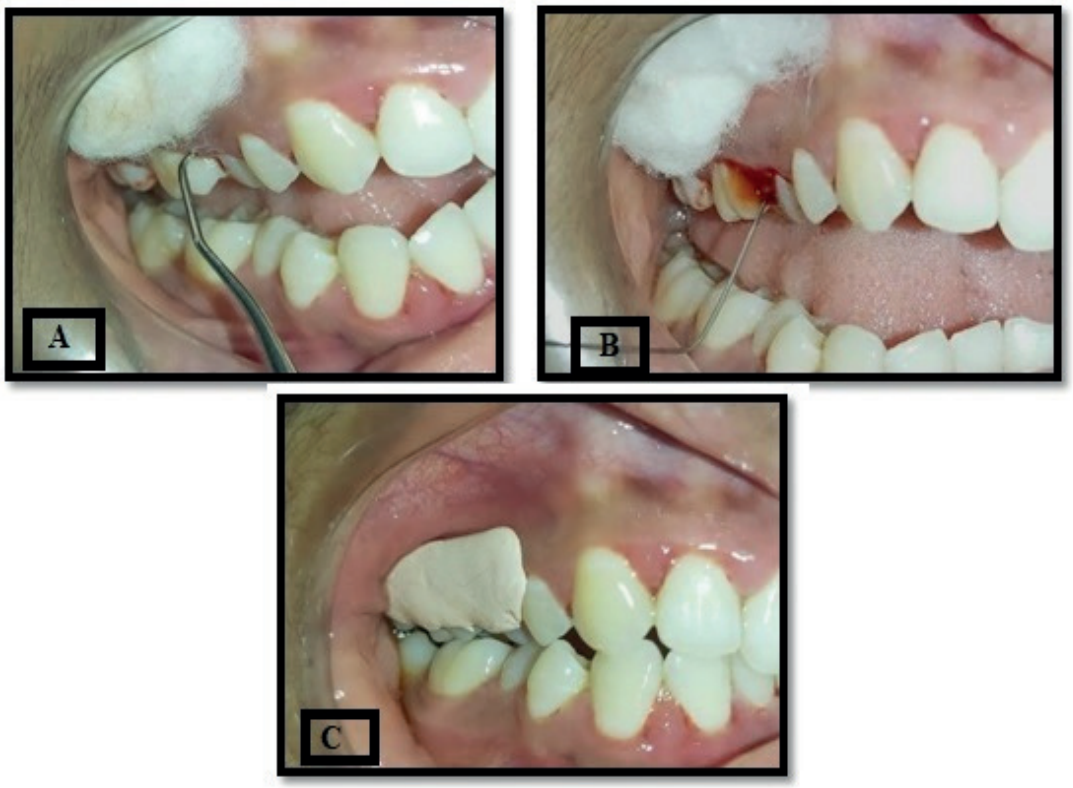


Figure.2- (A) Subgingival plaque (B) Drug delivery at the test site (C) Periodontal pack placed

Figure: 3 Samples stored in Eppendorf tubes with tris EDTA buffer

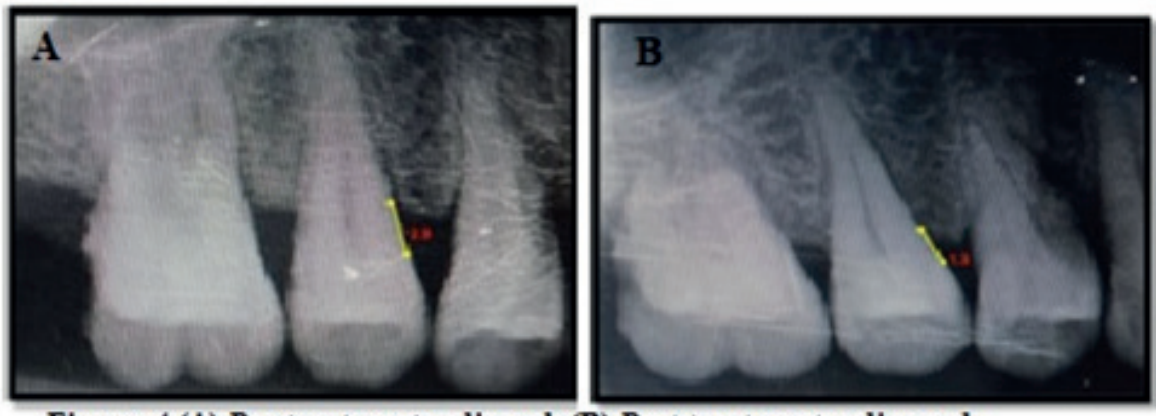


Figure. 4 (A)-Pre treatment radigraph (B)-Post treatment radiograph

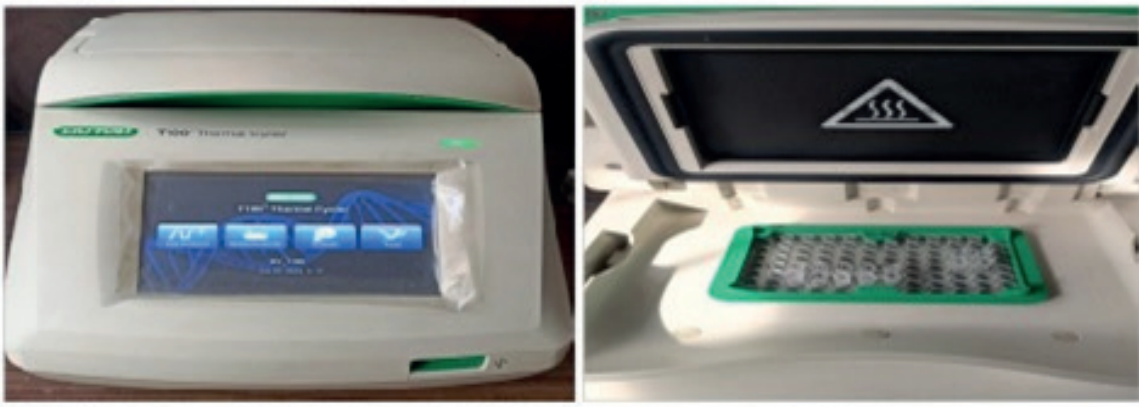


Figure.5- RT-PCR used for the study

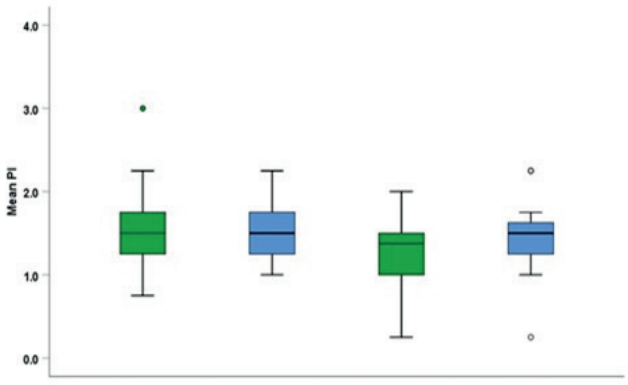


Figure.6-Comparison of mean PI scores between baseline and after 6 weeks

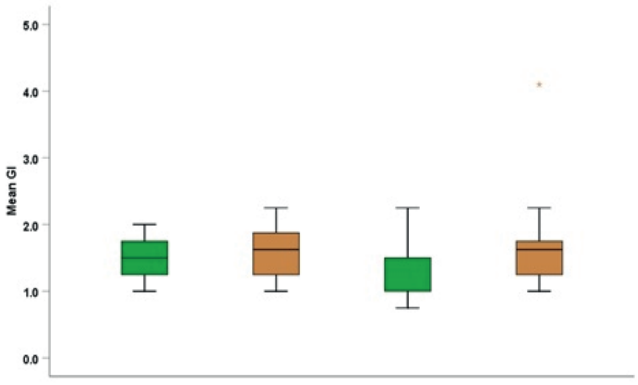


Figure.7-Comparison of mean GI scores between baseline and after 6 weeks

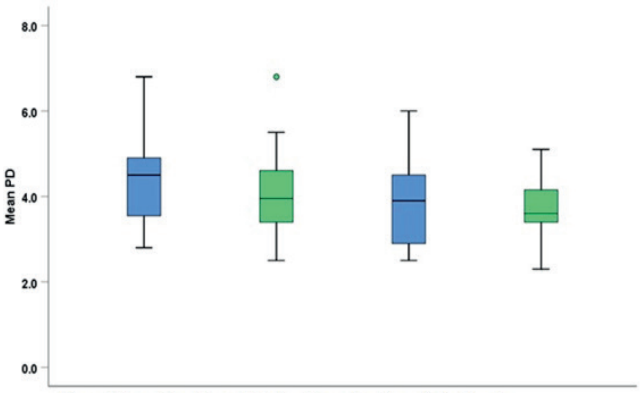


Figure.8-Comparison of mean PD values between baseline and after 6 weeks

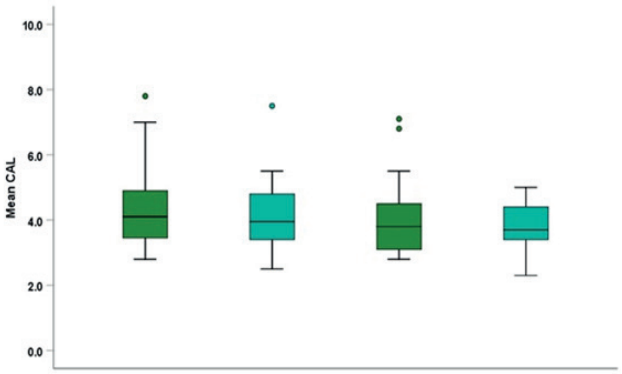


Figure.9-Comparison of mean CAL between baseline and after 6 weeks

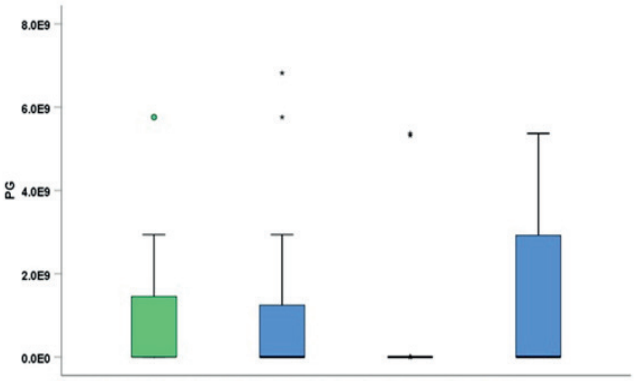


Figure.10-Comparison of P.gingivalis counts between baseline and after 6 weeks

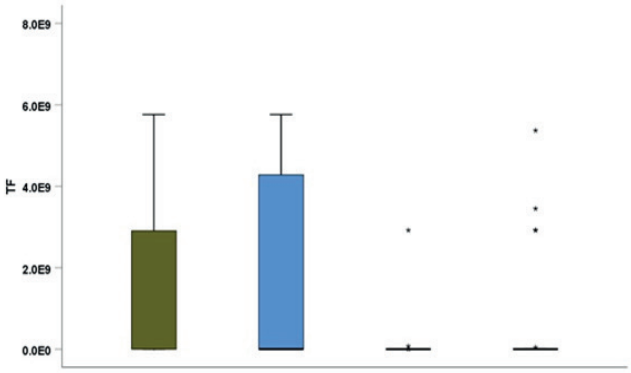


Figure.11-Comparison of T.forsythia counts between baseline and after 6 weeks

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