Original Article

Evaluation And Association Of Periodontal Status With Level Of P. Intermedia In Chronic Periodontitis Female Patients With And Without Oral Contraceptive Pills Following Non-Surgical Periodontal Therapy Using Quantitative Polymerase Chain Reaction: An Interventional Study

SARANSH (CORRESPONDING AUTHOR)[1] , PRIYANKA TANDON[2] , DIVYA DUBEY[3] , HIMANI SHARMA[4] , STUTI GUPTA[5] , PRACHI PATHAK[6]

[1]ASSISTANT PROFESSOR, DEPARTMENT OF PERIODONTOLOGY SCHOOL OF DENTAL SCIENCES, SHARDA UNIVERSITY , GREATER NOIDA, U.P. srnsh92@gmail.com
[2]ASSISTANT PROFESSOR, DEPARTMENT OF PERIODONTOLOGY I.T.S. DENTAL COLLEGE , GREATER NOIDA, U.P.
[3]ASSISTANT PROFESSOR, DEPARTMENT OF PERIODONTOLOGY Dr. G.D. POL FOUNDATION'S Y.M.T. DENTAL COLLEGE & HOSPITAL, MUMBAI
[4]ASSISTANT PROFESSOR, DEPARTMENT OF PERIODONTOLOGY SCHOOL OF DENTAL SCIENCES, SHARDA UNIVERSITY , GREATER NOIDA, U.P.
[5]ASSISTANT PROFESSOR, DEPARTMENT OF PERIODONTOLOGY SCHOOL OF DENTAL SCIENCES, SHARDA UNIVERSITY , GREATER NOIDA, U.P.
[6]ASSOCIATE PROFESSOR , DEPARTMENT OF PAEDODONTICS I.T.S. DENTAL COLLEGE , GREATER NOIDA, U.P.
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Abstract

Aim : The aim of the present study was to detect and correlate the levels of Prevotella Intermedia with clinical parameters after nonsurgical periodontal therapy (NSPT) in chronic periodontitis female patients with or without oral contraceptive pills, using quantitative polymerase chain reaction (Q-PCR) method.

Materials and Methods : Sixty female patients equally divided into three groups,
i.e. Periodontally health female patients (Group –I), females with Chronic Periodontitis (CP) without taking oral contraceptive pills(OCP) (Group-II), females with Chronic Periodontitis (CP) taking oral contraceptive pills(OCP) (Group-III) and were correlated for the presence of P. Intermedia in the respective groups. PPD, CAL, and saliva samples for microbiological evaluation were assessed at baseline, 1-, and 3-month post-NSPT.

Results : Significant reduction of PPD was found 2.4 ± 0.48mm versus 1.4 ± 0.37 mm in Group I, 4.8 ± 0.74 versus 2.8 ± 0.53 mm in Group II, and 6.6 ± 0.48 mm versus 4.4 ± 0.39 mm in Group III post-NSPT at 3 months. Similarly, a notable reduction of CAL was exhibited in both Group II (5.4 ± 0.65 vs. 3.1 ± 0.42 mm) and Group III (6.6 ± 0.48 vs 3.6 ± 0.35 mm) patients after

NSPT at 3 months. A greater reduction of P. Intermedia concentrations was observed in both Group II and Group III at 3-month post-NSPT.

Conclusion : The substantial improvement of clinical parameters was found to be in correlation with the load of P. Intermedia, which was reduced more in Group II than in Group III, emphasizing the applicability and sensitivity of Q-PCR method for its assessment.

Keywords : Chronic periodontitis, Oral contraceptive pills, Prevotella Intermedia, quantitative polymerase chain reaction

Introduction

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both. Ideally, periodontal therapy should eliminate inflammation, arrest progression of periodontal disease, improve esthetics, and create an environment conductive to maintenance of health.[1]

Nonsurgical periodontal therapy (NSPT) is the cornerstone of periodontal therapy and the first recommended approach to the control of periodontal infections. It is also known as "Cause-related therapy," "Phase I therapy or Etiotrophic
phase," and "Initial therapy." It is defined as "plaque removal, plaque control, supragingival and subgingival scaling root planing (SRP), and adjunctive use of chemical agents." Although NSPT has evolved over the years, it is still considered to be the "gold standard" to which other treatment methods are compared. [2]

Chronic periodontitis (CP) is a multifactorial disease which majority includes Gram-negative putative pathogens in dental biofilm.[3]

Hormonal contraceptives are drugs used to prevent pregnancy that can also be used in specific situations as family planning, menstrual cycle regularization, reduction in the incidence of ovarian cysts, etc. Among the hormonal contraceptive alternatives, there are many birth control options including contraceptive pills, contraceptive patches, implants, injections, intravaginal, and intrauterine delivery. Generally, in the composition of each contraceptive drug, there are two synthetic hormones, estrogens and progestins, which act by performing selective inhibition of pituitary function.[4]

In the oral cavity, this relationship has been associated with periodontal status, because the sexual steroids play significant roles in modulating inflammatory response of periodontal tissues and may alter the response to oral these structures during different phases of life, including puberty, menstruation, pregnancy, menopause and post-menopausal.[5] Increased sex hormone levels in serum could induce tissue destruction by activating matrix metalloproteinases proteins (MMPs) . The use of hormonal contraceptives by women at their reproductive life has been considered to influence periodontal disease progression .[6]

The use of oral contraceptives increases the levels of female sex hormones present in the sub-gingival environment which could lead to periodontal disease. It has been found that females using oral contraceptives have greater tendency for gingival bleeding, loss of attachment and greater periodontal pocket depth due to increased cellularity and increase in gingival cervicular fluid, along with increase in Prevotella species and almost 16 times increase in Bacteroides species level than normal gingival flora. [7]

Prevotella intermedia, a gram-negative, black-pigmented, obligate anaerobic rod, has been implicated in many forms of human periodontal disease, including chronic periodontitis, early-onset periodontitis , acute necrotizing ulcerative gingivitis , and pregnancy gingivitis .[8]

To establish causality, amplification of nucleotide sequences in hypervariable regions of 16s ribosomal nucleic acids, specially possessed by each bacterial species should be analyzed. The evidence should include fewer or no copies of RNAs in the inactive state of a disease. [9] Polymerase chain reaction-based assay with specific primers is considered to be a useful tool for precise quantification of individual species and detecting etiology of CP. Many studies have been reported to detect microbial concentrations using subgingival plaque but very few have used saliva as a sample. The number of studies incorporating quantitative polymerase chain reaction (Q-PCR) to investigate salivary bacteria are limited too.[10] While taking PCR into consideration, a comprehensive sampling and primer-based Q-PCR assay are sensitive than culture techniques, and it maximizes the likelihood of detection of P.Intermedia . In case of detectable salivary pathogen even after single visit of NSPT, further therapy or changes in treatment plan is indicated until its complete eradication from the samples is achieved. Thus, the study was carried out to assess levels of P. Intermedia in saliva and correlate it with the clinical parameters following NSPT among periodontally healthy, CP with and without OCP in female patients using Q-PCR method.

Materials and methods

The study population comprised of 60 female patients, which were divided equally into 3 groups with in the age range of 22-35 years visiting the outpatient department of periodontics in our institute. The study was approved by institutional ethical committee (IEC) which was completed within a time period of 24 months . Before the initiation of the study, an informed consent was obtained from the patients. A dental and medical history was recorded for the selected patients and intra-oral examination was conducted.

Inclusion criteria were female patients (22-35 years) with presence of atleast 20 natural teeth with moderate to severe clinical attachment loss with >30% of sites involved and the female patients who are taking oral contraceptive pillls .

Pregnant women or lactating mothers, tobacco chewers and cigarette smokers, patients who had undergone any periodontal therapy in the past 6 months, those were on antibiotics, those had anti-inflammatory therapy, and those having any systemic diseases were excluded from the study.

Female Patients were then categorized into following three groups :

Group I - comprised twenty females who are systemically and periodontally healthy individuals who presented teeth with periodontal pocket depth (PPD) ≤3 mm, clinical attachment loss (CAL) = 0, with no evidence of radiographic bone loss.

Group II - comprised twenty females who are systemically healthy patients with generalized moderate-to-severe CP without taking OCP, having PPD of ≥5 mm and CAL ≥5 mm, presenting bleeding on probing (BOP), and radiographic evidence of bone loss .

Group III - comprised twenty females with generalized moderate-to-severe CP patients who were taking OCP (as obtained from their medical history), with PPD of ≥5 mm
and CAL ≥5 mm that were positive for BOP, presenting radiographic evidence of bone loss .

5 ml of non-stimulated saliva was collected in Eppendorf tubes by guiding patients to spit into the tubes. Samples were collected at baseline and after scaling and root planing (SRP) is done at the end of 1st and 3rd month. Collected samples were then stored at −80°C until assayed.

The analysis of levels of P. Intermedia by Q-PCR in saliva was done at baseline, post 1 and 3 months of NSPT. DNA extraction from saliva was done using the following protocol: 25 µl of saliva was added to 500 µl phosphate-buffered solution and centrifuged at 10,000 rpm for 5 min (supernatant was discarded). To the remaining pellet, 0.5

M 50 µl NaoH was added and incubated at room temperature for 30 min. 50 µl of 1M Tris HCl was then added and vortexed to which 400 µl distilled water was added and stored at −20°C until further analysis.

Q-PCR for P. Intermedia was performed using following primers (5`-3`).

Forward: 5 CATAGATATCACGAGGAACTCCGATT-3`.

Reverse: 5`AAACTGTTAGCAACTACCGATGTGG-3`.

The Q-PCR amplification was done in PCR Thermocycler machine with denaturation at 95°C for 5 min, annealing temperature of 60°C for 1 min, and polymerization temperature of 74°C for 2 min. Clinical parameters were recorded along with the detection of P. Intermedia in saliva samples at baseline, 1 and 3 months of NSPT.

Stastical Analysis

The presence or absence of P.Intermedia in saliva samples was performed using Fisher's exact test and Z-test of proportion between independent samples, respectively. The statistical comparison of parameters within groups before and after treatment was performed using paired t-test. The post hoc analysis was performed using Tukey's HSD test. Statistical significance was tested at 5% level, and all the analyses were performed using computer software (SPSS version 20.0, IBM Inc. Armonk, NY, USA).

Results

In this interventional study, NSPT was carried out and its influence over clinical parameter and microbiological assessment of P.Intermedia load in saliva was conducted with the advanced and most sensitized Q-PCR technique. To evaluate the inflammatory status among the groups, all the clinical parameters were assessed at baseline, 1, and 3 months post-NSPT.

Results thus indicated a significant reduction in the means of PPD (Fig 1) and CAL (Fig 2) after a comprehensive NSPT using one-way ANOVA. (Table 1)

To evaluate the effect of NSPT on P. Intermedia, saliva samples from Group I, Group II, and Group III were analyzed to assess if complete elimination of P. Intermedia could be achieved at baseline, 1, and 3 months post-NSPT. None of the samples from Group I patients presented detectable amount of P. Intermedia at baseline. Among Group II, P. Intermedia was detected in 16 patients, which reduced to 3 after 1 month and only one patient was found to be positive for P.Intermeida detection, 3-month post-NSPT. In Group III, P. Intermedia was detected in all the 18 patients before SRP and the number of patients which retained P. Intermeida post 1- and 3-month recall were 4 and 2, respectively . Intragroup analysis showed a greater reduction of P. Intermedia in both Groups II and III, while no statistically significant reduction was observed between Group II and III at 3-month post-NSPT.

The analysis of P. Intermedia levels by Q-PCR showed the following readings. According to densiometric analysis, the concentration of DNA marker at the 410 bp position was 10 ng/µl and concentrations of P. Intermedia were 4 ng/µl pre-NSPT, 4 ng/µl post 1 month of NSPT, and 4 ng/µl post 3 months of NSPT with respect to saliva sample number 1. Similarly, for sample 2, concentration of DNA marker at the 410 bp position was 10 ng/µl and concentrations of P. intermedia were 4 ng/µl pre-NSPT, 1 ng/µl post 1 month of NSPT, and 0 ng/µl post 3 months of NSPT. For sample 3, concentrations of P. Intermedia were 25 ng/µl pre-NSPT, 0 ng/µl post 1 month of NSPT, and 0 ng/µl post 3 months of NSPT. For sample 4, concentrations of P. Intermedia were35 ng/µl pre-NSPT, 4 ng/µl post 1 month of NSPT, and 01 ng/µl post 3 months of NSPT. For sample 5, concentrations of P. intermedia were 25 ng/µl pre-NSPT, 4 ng/µl post 1 month of NSPT, and 01 ng/µl post 3 months of NSPT.(Fig 3)

Discussion

Oral Contraceptive pill (OCP) contains progesterone and estrogen. High level of progesterone increases the blood flow to the gingival tissue and causes gingiva to be more sensitive and vulnerable to irritation and swelling. Vasodilatation
and increased capillary permeability is caused by the additive effect of estrogen and progesterone which further leads to increased migration of fluid and white blood cell out of blood vessels. The change in progesterone and estrogen levels affects the immune system as well as the collagen production in the gingiva. Both of these conditions reduce the body's ability to repair and maintain
gingival tissues.[11] Moreover, OCP users also showed a higher prevalence of P. gingivalis, P. intermedia, and A. actinomycetemcomitans as compared to non-users .[12]

When prevalence and severity of periodontal disease in female patients who are taking OCP , it was found that the increased severity of periodontal disease . On the other hand, the clinical improvements in parameters were found in females to correlate with the good systemic health and the microbial burden, especially of the periodontopathic microbes. There was a significant improvement in clinical parameters such as PPD and CAL after SRP in both the groups, but the Group II showed better improvement as compared to Group III. Many studies have reported that female patients taking OCP have an inherent increased risk for initiation of inflammation and delayed tissue healing .[11] Hence, it was probable to find lesser improvement in clinical parameters after therapy in female patients who are taking OCP as compared to female patients who are not .

A physically powerful association of the putative pathogens, especially P. Intermedia, P. gingivalis , and A. actinomycetemcomitans with that of the disease has been established in the previous study.[13] The results of our study were in accordance with Saygun et al.[14] & He et al.[15] where P. Intermedia count was significantly higher in CP patients than periodontally healthy participants. NSPT is capable of significantly reducing the number of these subgingival microorganisms and therefore considered as a cornerstone of periodontal therapy. Furthermore, Haffajee et al.[16] found reduction in gingival redness along with the reduction of fourty bacterial species including A. actinomycetemcomitans,P. gingivalis, P. intermedia, and T. denticola and mean gain of clinical attachment level after NSPT. Periodontal pathogen reduction along with a significant improvement in clinical parameters, thus necessitates microbial monitoring along with clinical monitoring to provide guidance for planning treatment strategies.

There are some literature which show that limited studies on the detection of salivary pathogens & their co-relation with clinical parameters in Chronic peridontitis patients . Advantages of utilizing saliva as a biomarker over plaque have been observed by Yamanaka et al. states tha as the microbial diversity and
richness were found to be reduced in plaque samples but not in saliva before as well as after surgical periodontal therapy.[17] Higher detection rates of P. Intermedia in saliva samples and possibilities of amplification of nonviable bacterial cells by PCR as compared to culture methods, Q-PCR method was used for analysis of saliva samples in our study.[18] When the reduction in P. Intermedia count among Group II and Group III patients was compared, the difference was negligible. It has been emphasized in the literature that the patients presenting significantly a higher number of P. Intermedia (target organisms) are indicated as diseased, while mere presence of these organisms will not suffice.

Zakaryia et al. showed that the use of contraceptive pills can affect the periodontium resulting in gingival diseases especially by using of newer generation .[19] Several mechanisms have been suggested for this heightened response in gingival tissues. It has been shown that human gingiva contains receptors for progesterone and estrogen.[20] Existence of these receptors might provide evidence that periodontal tissues are a target for the gestational hormones. Progesterone causes increased vascular permeability and an increased synthesis of prostaglandin. Prostaglandin E, a mediator of inflammation, appears to rise significantly with increasing levels of sex hormones.[21]

In high dose, contraceptives pills produce a hypertrophic gingivitis, bleeding and pregnancy type epulis, marked gingival erythema and in therapeutic which may produce gingivitis with an enlarge in gingival exudate and enhance the amount of inflammation in papillae .[22]

Hence, the chances of elimination in P.Intermedia after therapy are comparatively lesser in CP patients with OCP than in patients of CP without OCP. Thus, patients found positive for target pathogen P.Intermedia even after 3 months of NSPT was considered for further continuation of therapy and should be kept under observation.

Although mechanical therapy effectively has been proved to reduce microbial load when evaluated using DNA probes, its complete elimination is difficult. Thus, if chairside diagnostic test is included to monitor changes in microbial levels, it would help in estimating type and duration of therapy provided, need
of any adjunctive as well as prediction of outcome of a planned therapeutic strategy. Limitations were smaller sample size and analysis of P. Intermedia load alone. However, a larger sample size with longitudinal evaluation and complete microbial array of PCR would have given a proactive approach for diagnosis, prevention, and treatment planning of periodontal disease.

Conclusion

Several research and clinical studies obtainable in the past and now a day have concluded that oral contraceptive pills have certain effects on oral health. The judgment of dependent dose special effects led to the improvement of new production of oral contraceptive pills with minimum dose content in current years
, although a small number of studies have been done to explore their special effects on oral health, the evidence powerfully provide evidence that they will not preteens health risks. So, women taking contraceptive pills have a low danger for having periodontal and gingival disease.

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Table 1 : Descriptive statistics for various parameters according to groups

Parameters	Group-I (n=20)	Group –II (n=20)	Group-III (n=20)	P
Pocket probing depth (PPD)				
Pre-t/t	**2.4±0.48**	**4.8±0.74**	**6.6±0.48**	**<0.0001**
Post t/t	**1.4±0.37**	**2.8±0.53**	**4.4±0.39**	**<0.0001**
Clinical attachment level (CAL)				
Pre-t/t	**0**	**5.4±0.65**	**6.6±0.48**	**<0.0001**
Post t/t	**0**	**3.1±0.42**	**4.6±0.35**	**<0.0001**
Prevotella Intermedia				
Pre-t/t	**-**	**16**	**18**	**-**
Post t/t -1 month	**-**	**3**	**4**	**-**
Post t/t-3 Month	**-**	**1**	**2**	**-**

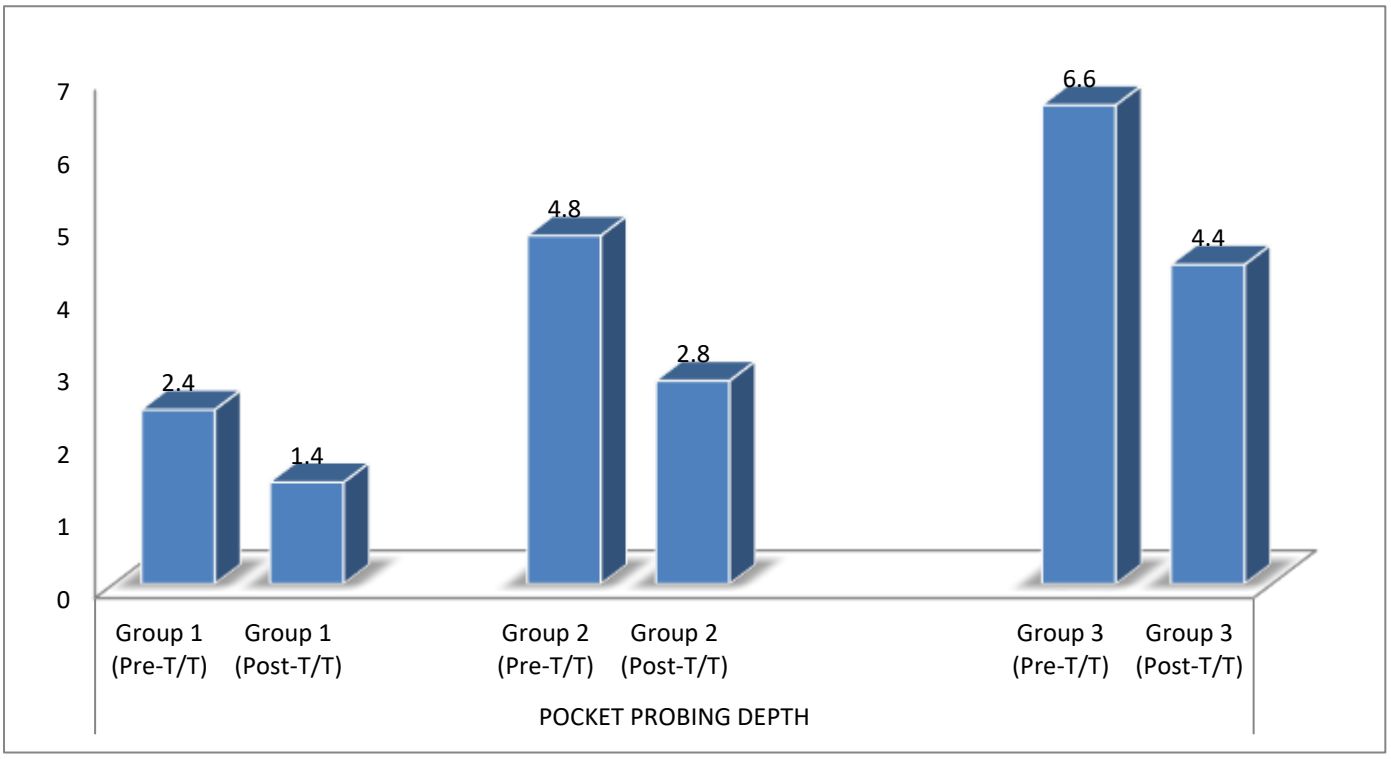

Fig 1. Pocket probing depth level

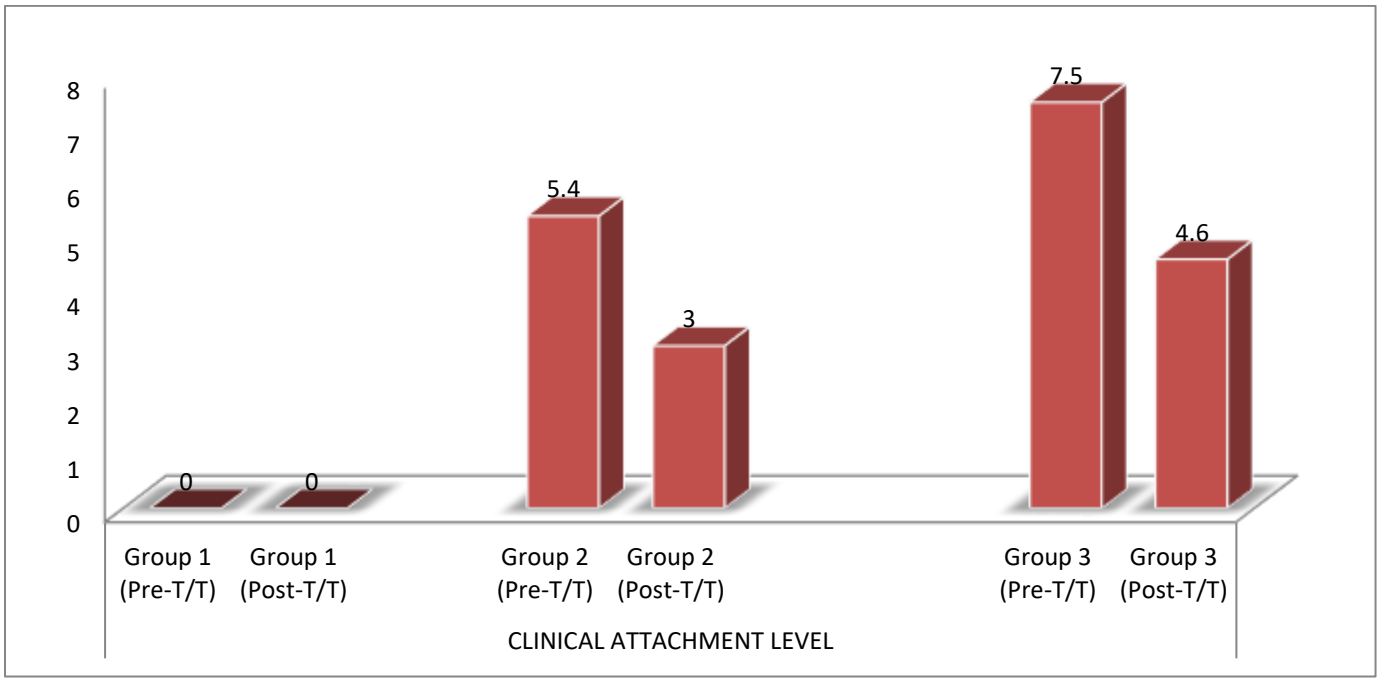

Fig 2. Clinical Attachment Level

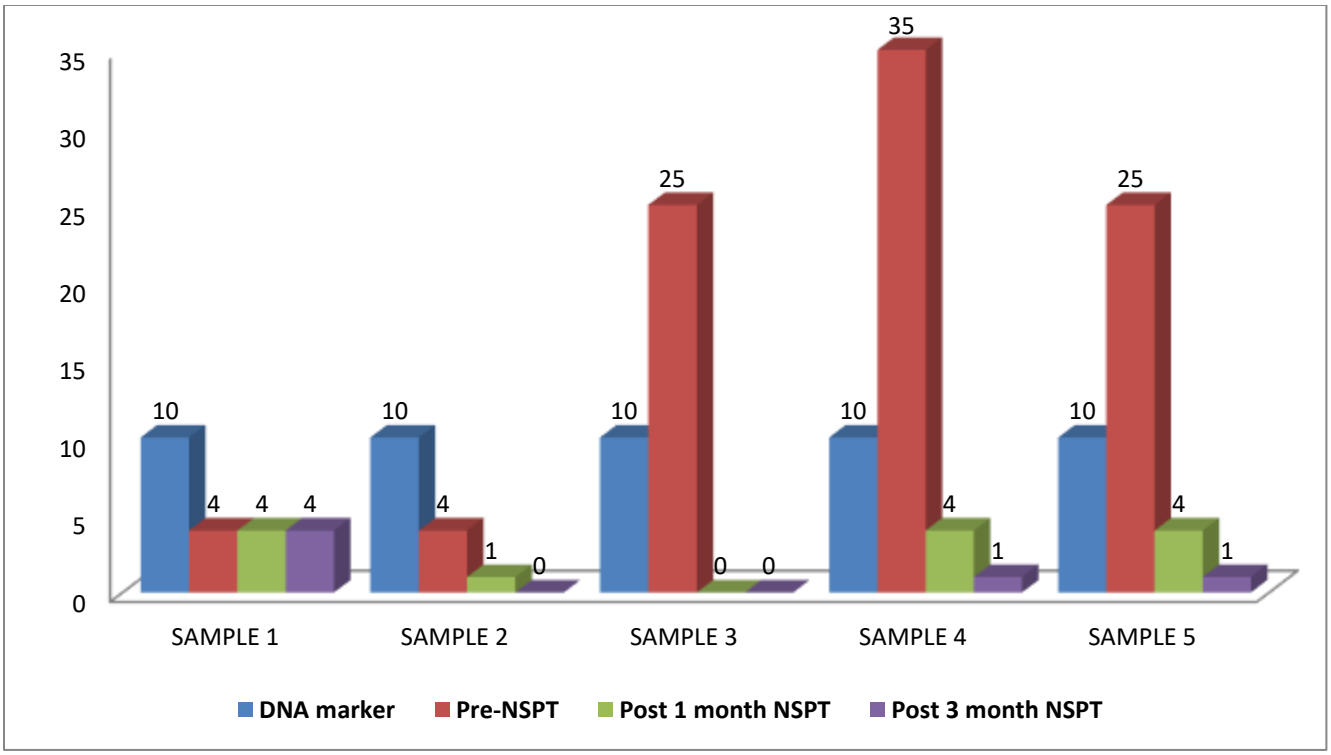

Fig 3 : Bar chart representing Prevotella Intermedia detection at baseline, post 1 month and post 3 months of nonsurgical periodontal therapy with respect to concentration of deoxyribonucleic acid marker.
DNA - deoxyribonucleic acid;
NSPT- Non-surgical periodontal therapy