EFFECT OF PHOTODYNAMIC THERAPY AS AN ADJUNCT TO NON-SURGICAL PERIODONTAL THERAPY ON GINGIVAL CREVICULAR FLUID INTERLEUKIN-6, INTERLEUKIN-8 & INTERLEUKIN -10 LEVELS IN CHRONIC PERIODONTITIS : A RANDOMIZED CONTROLLED TRIAL

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LIST OF ABBREVIATIONS

Abbreviation	Full form
IL-1	Interleukin-1
IL-6	Interleukin-6
IL-8	Interleukin-8
TNF-alpha	Tumour necrosis factor-alpha
GCF	Gingival crevicular fluid
IL-4	Interleukin-4
IL-10	Interleukin-10
IL-13	Interleukin-13
TGF-β	Transforming growth factor -β
TIMP	Tissue inhibitors of matrix metalloproteinases
PMN	Polymorphonuclear leukocytes
IL-1α	Interleukin-1alpha
IL-1β	Interleukin-1beta
INF-γ	Interferon- gamma
IL-17	Interleukin-17
RANK	Receptor activator of nuclear factor kappa
RANKL	Receptor activator of nuclear factor kappa ligand
SRP	Scaling and root planing
NSPT	Non-surgical periodontal therapy
PDT	Photodynamic therapy
LED	Light emitting diode
TBO	Toluidine blue O
CP	Chronic Periodontitis
hs-CRP	High-sensitivity C-reactive protein
MCP-1	Monocyte chemoattractant protein-1
sP-selectin	Soluble P-selectin
sVCAM-1	Soluble vascular adhesion molecule-1

sICAM-1	Soluble intercellular adhesion molecule-1
ELISA	Enzyme-linked immunosorbent assay
PI	Plaque index
GI	Gingival index
PPD	Probing pocket depth
CAL	Clinical attachment level
TAIL-6	Mean total amounts of IL-6
TAIL-8	Mean total amounts of IL-8
GAgP	Generalised aggressive periodontitis
DNA	Deoxyribose Nucleic Acid
MMP-8	Matrix metalloproteinases-8
BOP	Bleeding on probing
GML	Gingival margin level
TRG	Triglyceride
IL-18	Interleukin-18
PCR-RFLP	Polymerase chain reaction-restriction fragment length polymorphisms
IL-35	Interleukin-35
MMP-3	Matrix metalloproteinases-3
IL-34	Interleukin-34
IL-1ra	IL-1 receptor antagonist
b-FGF	Basic fibroblast growth factor
G-CSF	Granulocyte colony-stimulating factor
DSL	Diode soft laser
a2M	a-2 macroglobulin
CRP	C-reactive protein
GM-CSF	Granulocyte macrophage colony-stimulating factor
PS	Photosensitizer
GCP	Generalized chronic periodontitis
aPDT	Antimicrobial photodynamic therapy
PD	Probing depth
FMPS	Full-mouth plaque score
FMBS	Full-mouth bleeding score
GR	Gingival recession
RCAL	Relative clinical attachment level

Aa	Aggregatibacter actinomycetemcomitans
Pg	Porphyromonas gingivalis
Pi	Prevotella intermedia
Tf	Tannerella forsythia
Pn	Prevotella nigrescens
PCR	Polymerase chain reaction
GBI	Gingival bleeding index
LT	Laser therapy
PBI	Papillary Bleeding Index
SBI	Sulcus bleeding index
MC	Minocycline microspheres
RAL	Relative Attachment Level
ICG	Indocyanine green
SFFR	Sulcus fluid flow rate
OHI-S	Oral Hygiene Index-Simplified
T2DM	Type 2 diabetes mellitus
HbA1c	Glycated hemoglobin
UNC-15	University of North Carolina-15
CEJ	Cementoenamel junction
TMB Substrate	3,3’,5,5’-Tetramethylebenzidine
ANOVA	Analysis of variance
LPS	Lipopolysaccharide
MB	Methylene blue

Introduction

Periodontitis, is defined as a bacterially induced inflammatory disease of the tooth supporting (periodontal) tissues. The complex interaction of host defense mechanisms, microbial agents, genetic & environmental factors leads to clinical expression of the diseases. If left uncontrolled, the disease in their severe forms precipitate progressive destruction ultimately leading to tooth loss & edentulism. Over 700 bacterial species are harboured in the oral cavity that are organized in a complex polymicrobial biofilm, which plays a major role in the etiology of periodontitis. A "microbial shift" in the biofilm results in destructive immunological host responses and disturbed homeostasis. This dysbiosis may cause periodontitis, which is characterized by gingival inflammation associated with bleeding, periodontal pocket formation, destruction of periodontal ligament and tooth loss.

Bacteria mostly cause the observed tissue destruction indirectly by activating various components of the host defense systems in such a manner that destruction proceeds. It is perplexing that protection as well as defense which are provided by the same host systems, are responsible for destruction.[1] After host-bacterial interaction, an acute inflammatory response begins which then progresses into a chronic stage dominated by B lymphocytes and macrophages, following intense T lymphocytes stage. Transition between these stages as well as accumulation of immune cells and their differentiation in the inflammatory site are mediated by "Cytokines."[2] Cytokines are low molecular weight water-soluble inflammatory proteins secreted by hematopoietic and non-

hematopoietic cells that transmit signals to other cells and are contributed in many biologic processes such as systemic and local inflammatory responses, hematopoiesis and wound healing. Pleiotropic effects shown by cytokines on different target cells by regulating cell activation, proliferation and function. Cytokines may serve as a critical determinant of tissue destruction in many chronic inflammatory diseases like inflammatory osteoarthritis, rheumatoid arthritis and periodontitis.[2] The complex cytokine network that mediates the immune response includes pro-inflammatory cytokines, anti-inflammatory cytokines and specific cytokine receptors.

Inflammatory cytokines which are produced during inflammatory responses have been associated with the onset or progression of tissue insult. It is these pro-inflammatory cytokines that can modulate and affect homeostasis of both hard and soft tissues.[3] Pro-inflammatory cytokines comprising Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), and tumour necrosis factor-alpha (TNF-alpha) form an environment that helps disease progression by enhancing the bactericidal capacity of phagocytes, recruiting additional innate cell populations to sites of infection, inducing dentritic cell maturation and direct the subsequent specific immune response to the invading microbes. Various studies have demonstrated that inflammatory cytokines are responsible for early responses to bacterial aggression and the increase in their levels in GCF (Gingival crevicular fluid). Anti-inflammatory cytokines either block this process or suppress the intensity of the cascade. For example, Interleukin-4 (IL-4), Interleukin-10 (IL-10), Interleukin-13 (IL-13) and transforming growth factor (TGF) -β suppress the production of IL-1, TNF, chemokines such as IL-8, and vascular adhesion molecules.[4]

IL-6 is an important cytokine involved in the regulation of host response to tissue injury and infection. In response to inflammatory challenges it is produced by a variety of cells, such as monocytes, fibroblasts, osteoblasts, and vascular endothelial cells. It plays an important role in B-cell differentiation and in T-cell proliferation,[5] acceleration of bone resorption by increasing osteoclast formation.[6] However, its role in increasing the production of tissue inhibitors of matrix metalloproteinases (TIMP)[7,8] has also been reported, likewise suppresses IL-1 expression.[9] IL-8 belongs to the interleukin-8 supergene family that includes small chemotactic peptides with analogous activity for specific types of leukocyte populations.[5,10] This cytokine is induced and secreted by many cells, such as monocytes, lymphocytes, fibroblasts, epithelial, and endothelial cells as well as by synovial cells. In inflammatory regions, IL-8 attracts and activates polymorphonuclear leukocytes (PMN), induces the adhesion of PMN to endothelial cells and their transendothelial migration as well as the release of granule enzymes from these cells.[5] IL-8 being a potent chemoattractant for neutrophils exerts various effects on neutrophil activity, including stimulation of granule exocytosis and release of myeloperoxidase, elastase and b-glucuronidase. As unquestionably, neutrophils play a vital role in the development of inflammatory injury, IL-8 is of considerable importance for neutrophil induced tissue destruction. The role of IL-8 in the pathological processes within periodontal tissues has been explored, and it has been specified that excessive IL-8-mediated processes within the periodontal tissues may contribute to local periodontal tissue destruction.[11]

In a complex network of pro- and anti-inflammatory cytokines acting in the inflamed periodontal tissues, a cytokine with anti-inflammatory effects is IL-10. IL-10 is a regulatory cytokine, which not only limits inflammatory responses by inhibiting the expression of proinflammatory cytokines (e.g. IL-1α, IL-1β, IL-6, TNF-α), but also

upregulates the recruitment and activation of B cells.[2,4] By controlling the B-cell lesion and downregulating the T helper 1 response, it is suggested to play a role in controlling the progression of periodontal disease.[4] IL-10, demonstrates wide immunoregulatory effects by reducing Interferon-gamma (INF-γ) and Interleukin-17 (IL-17) from T cells and also inhibiting bone resorption via preventing RANK-RANKL connection.[2]

It is commonly known that the success of periodontitis treatment depends not only on removal of the microbial biofilm, but also eliminating periodontopathogens and their toxic products such as lipopolysaccharide from the dental root surface and periodontal soft tissues, as well as neutralization of host pro-inflammatory cytokines. Though scaling and root planing (SRP) is considered to be the gold standard for the non-surgical treatment of periodontal disease but it is ineffectual in completely eliminating subgingival bacteria and calculus. These limitations could be attributable to deep narrow intrabony defects, furcation involvement, presence of anatomical variations like root curvatures and invaginatus, which can make it difficult for SRP to completely remove bacterial deposits and biofilms from the root surface. Hence, after treatment the periodontal healing process can be affected by the remaining bacterial reservoir.[12] To overcome this problem, non-surgical periodontal therapy (NSPT) may be supported by the use of local or systemic antibiotics. However, headache, dizziness, gastrointestinal disturbances and allergies are some of the side effects which are associated with systemic antimicrobial therapy. Moreover, development of resistant microorganisms may occur with the frequent use of antibiotics. Additionally, the insufficient concentration of the drug in the sulcular fluid and presence of the periodontal pathogens in a biofilm environment, which protects them from antibiotic action are the major drawbacks of systemic antibiotics for treating periodontal disease. Thus, to avoid some of these pitfalls, local delivery of antimicrobial agents directly into periodontal pockets

has been put forward as an alternative to systemic antibiotics. However, application of this method can be technically difficult in patients with generalized periodontitis and multiple sites of deep pockets.[13] To directly address this alarming problem of antibiotic resistance, promotion of new and effective therapeutic treatment strategies is crucial.

One such a new, novel, non-invasive treatment approach for infection control is Photodynamic therapy (PDT) which is also called as photoradiation therapy, phototherapy or photochemotherapy.[14] Von Tappeiner in 1904 coined the term photodynamic to describe oxygen-dependent chemical reactions induced by photosensitization which could inactivate bacteria.[15] PDT includes the combination of visible light, usually through the use of a diode laser and a photosensitizer. The photosensitizer is a compound that is capable of absorbing light of a specific wavelength and transforming it into useful energy. Each component is harmless by itself, but when combined they can selectively destroy cells by producing lethal cytotoxic agents.[14] It is based on the principle that a photosensitizer (i.e. a photoactivatable substance) binds to the target cells and is activated by light of a suitable wavelength. Following activation of the photosensitizer through the application of light of a certain wavelength, singlet oxygen and other very reactive agents are produced that are extremely toxic to certain cells and bacteria. Sources of light include a range of lasers, gallium – aluminium-arsenide diode lasers (630-690, 830 or 906 nm), helium-neon lasers (633 nm), and argon laser (488-514nm), the wavelength of which ranges from the red of helium-neon laser to the infra-red area of diode lasers or from visible light to the blue of argon lasers. Non-laser light sources includes light emitting diode (LED) and light cure units.[16]

A photosensitizer, which is a dye material, absorbed by the microorganism, cell or tissue allowing it to interact with the light. The photosensitizers used for medical purposes belong to the following basic structure:

1. Tricyclic dyes with different meso atoms. Eg: Acridine orange, proflavine, riboflavin, toluidine blue O, methylene blue, fluorescein and erythrosine.

2. Tetrapyrroles. E.g.: Porphyrins and derivatives, chlorophyll, phylloerythrin and phthalocyanines.

3. Furocoumarins. E.g.: Psoralen and its methoxyderivatives, xanthotoxin and bergaptene.

In antimicrobial PDT, mostly used photosensitizers are toluidine blue O (TBO) and methylene blue. Both have similar chemical and physicochemical characteristics. The positive charge seems to promote the binding of the photosensitizer to the gram-negative bacterial membrane leading to its localized damage, resulting in an increase in its permeability.[16]

Upon analysis of the data from several controlled clinical studies, the adjunctive use of PDT to SRP resulted in greater clinical attachment level gains, reduction in bleeding on probing and probing pocket depths in the treatment of patients with periodontitis.[3,12,15,16] However, a systematic review conducted by **Azarpazhooh et al. (2010)** concluded that PDT as an independent therapy or as an adjunct to SRP was not superior to control treatment than SRP.[16,17] In a study by **Moreira et al. (2015),** patients treated with aPDT as an adjunct to SRP showed significant reduction of IL-1β as compared to SRP alone. However, **Pourabbas et al. (2014)** in a clinical trial showed comparable IL-1β levels with and without aPDT. Therefore, the role of PDT as an adjunct to SRP appears to be controversial in the reduction of inflammatory markers.[3]

Usually, diagnosis of periodontal diseases is based almost exclusively on clinical parameters; however, increasing amounts of data from various studies specified that the intensity of inflammation as well as destruction within periodontal tissues can be assessed objectively by analysis of GCF components, because several inflammatory and immune mediators associated with periodontal destruction have been recognized in GCF.[11]In light of the above facts, the present study is designed to analyze effects of PDT as an adjunct to NSPT on the GCF IL-6, IL-8, and IL-10 levels in chronic periodontitis.

Aim and Objectives

Aim of the study

The present study aimed to evaluate the efficacy of PDT as an adjunct to NSPT on the GCF IL-6, IL-8 & IL-10 levels in chronic periodontitis.

Objectives of the study

Also glued to this aim were certain objectives:

1. To evaluate GCF IL-6, IL-8 & IL-10 levels in patients with CP.
2. To evaluate GCF IL-6, IL-8 & IL-10 levels, in patients with CP after NSPT.
3. To evaluate GCF IL-6, IL-8 & IL-10 levels, in patients with CP after application of PDT adjunctive to NSPT.
4. To compare GCF IL-6, IL-8 & IL-10 levels in patients with CP after NSPT alone versus use of PDT adjunctive to NSPT.

Review of Literature

Periodontal disease is initiated by pathogenic plaque biofilm which is characterized by bacterially-induced inflammatory destruction of tooth-supporting structures and alveolar bone. Mechanical scaling and root debridement have shown to be an effective treatment approach for periodontal disease. However, Limitations in conventional non-surgical periodontal therapy have led to the exploration of other treatment options to improve clinical outcomes.

Photodynamic therapy (PDT) has recently emerged as another augmentative therapy to scaling and root planing. Numerous studies have evaluated the effect of photodynamic therapy either as a primary mode of treatment or as an adjunct to mechanical debridement in periodontitis. Some results were non-definitive and in part contradictory with regards to the clinical and microbiological effects.

One approach for evaluating these effects is by monitoring the biomarkers in the GCF. Among many inflammatory and immune mediators identified in the GCF, cytokines have attracted particular attention and are suspected to be involved in both inflammation-related alteration and repair of the periodontal tissues. Few studies extendedly examined the localized effects of these inflammatory mediators associated with periodontal diseases, and proved the role of these mediators in innate and acquired immunity in periodontitis.

Considering the paramount literature available and for the ease of understanding, the review of literature has been segregated into four parts -

1. Studies on effect of NSPT on cytokines in Periodontitis
2. Studies on effect of PDT on cytokines
3. Studies on influence of NSPT and PDT (adjunctive therapy) on periodontitis
4. Studies on Interrelationship between IL-6, IL-8, IL-10 and periodontitis

1. Studies on effect of NSPT on cytokines in Periodontitis

Marcaccini MA et al. (2009)[18] compared chronic inflammation markers in control individuals and patients with periodontal disease and observed whether non-surgical periodontal therapy affected inflammatory disease markers after 3 months. Patients who were non-smokers, did not use any medication, and had no history or detectable signs and symptoms of systemic diseases were included in the study. Plasma and serum samples of 20 controls and 25 patients with periodontal disease were obtained prior to and 3 months after non-surgical periodontal therapy. Periodontal parameters (PD, CAL and BOP) and systemic parameters which including hematologic parameters, as well as the following inflammatory markers: IL-6, hs-CRP, CD40 ligand, monocyte chemoattractant protein (MCP)-1, soluble P-selectin (sP-selectin), soluble vascular adhesion molecule (sVCAM)-1, and soluble intercellular adhesion molecule (sICAM)-1 were assessed. An automated pocket probe was used for measuring periodontal parameters. Serum levels of total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol were measured with routine enzymatic assays. Fibrinogen was measured in citrated samples by the Clauss method. MCP-1, sICAM-1, IL-6, CD40 ligand, sP-selectin, and sVCAM-1 were measured in plasma by enzyme-linked immunosorbent assay (ELISA) kits. Plasma hs-CRP levels were evaluated using a kit

with specific high-sensitivity methodology in a spectrophotometer. They found no differences in the hematologic parameters of the patients in the control and periodontal disease groups. IL-6 concentrations were found to be higher in the periodontal disease group at baseline compared to the controls (P = 0.006). Significant reduction was found in all clinical parameters (P <0.001), and a decrease in circulating IL-6 and hs-CRP concentrations was observed 3 months after therapy (P = 0.001 and P = 0.006, respectively). These results highlighted the importance of treating periodontal disease to avoid elevated IL-6 and hs-CRP levels, which are increased in many systemic inflammatory diseases, such as atherosclerosis, rheumatoid arthritis, diabetes mellitus, and aneurysms.

Goutoudi P et al. (2011)[5] analysed the levels of interleukin-6 (IL-6) and interleukin-8 (IL-8) in GCF of patients with chronic periodontitis prior to and after nonsurgical periodontal therapy and/or surgical therapy for a period of 32 weeks. 12 patients with chronic periodontitis were recruited into this randomized longitudinal split-mouth interventional study, GCF samples were obtained from 24 non-diseased and 72 diseased sites using paper strips before as well as at 6, 16, and 32 weeks after non-surgical and surgical periodontal therapy. IL-6 and IL-8 levels were determined by enzyme-linked immunosorbent assay (ELISA). The periodontal status of each subject was assessed using plaque index (PI), gingival index (GI), probing pocket depth (PPD), and clinical attachment loss (CAL). Improvement was observed in all clinical parameters after periodontal treatment. As compared to diseased sites, mean IL-6 and IL-8 concentrations were significantly higher in non-diseased sites and increased significantly following treatment in diseased sites. Mean total amounts of IL-6 and IL-8 (TAIL-6, TAIL-8) did not differ significantly between diseased and non-diseased sites, while following therapy TAIL-8 levels were found to be decreased significantly. This

study concluded that, both treatment modalities improved significantly the clinical indices; this improvement was accompanied by a down regulation of the mean total amount of IL-8 in GCF. Nevertheless a strong relationship between IL-6 or IL-8 levels in GCF and periodontal destruction or inflammation was not found.

Rosalem W et al. (2011)[19] compared the effects of scaling and root planing on clinical parameters, the subgingival microbial profile, and expressions of GCF IL-1β, -4, and -8 and IFN-γ and elastase activity in patients with generalised chronic periodontitis (GCP) and generalised aggressive periodontitis (GAgP). Total 34 patients, GCP (n=20) and GAgP (n=14) were evaluated in this study. Clinical data, subgingival plaque samples and GCF were collected at baseline and 3 months following non-surgical periodontal treatment. Using checkerboard DNA-DNA hybridization, levels of 40 subgingival species were measured. GCF IL-1β, IL-4, and IL-8 and interferon- γ (IFN-γ) were analyzed using a multiplexed bead immunoassay, and elastase activity was measured using an enzymatic assay. For all clinical parameters, significant improvements were found in both groups after periodontal therapy as well as significant reductions in elastase activity in both deep and shallow sites from the GAgP group also in deep sites from the GCP group. As for changes in GCF biomarkers, in patients with GAgP there was found a trend with greater reductions in elastase activity and levels of IL-1β and IL-8 and IFN- γ. In the GCP group, IL-1β and IL-8 and IFN- γ increased in deep sites after therapy. Microbiologic data revealed significant reductions in proportions of red and orange complexes as well as an increase in proportions of Actinomyces species in both clinical groups. Only minor differences were found when clinical, microbiologic, and immunologic responses were compared between clinical groups after therapy.

Konopka L et al. (2012)[11] investigated the influence of scaling and root planing on amounts of IL-1β, IL-8 and MMP-8 in gingival crevicular fluid from patients with

chronic periodontitis, in relation to clinical parameters. Total 51 patients were enrolled in this study and are divided into 2 groups: 30 patients with generalized advanced chronic periodontitis were recruited for test group, while 21 periodontally healthy subjects were recruited for the control group. The clinical parameters included approximal plaque index, GI, PD and CAL were measured, while the amounts of IL-1β, IL-8 and MMP-8 in GCF were assessed by ELISA. Periodontal parameters and GCF humoral factor amounts were evaluated in the control group and in CP patients at baseline, 1 and 4 weeks after SRP treatment. The amounts of IL-1β, MMP-8 ($p < 0.001$) and IL-8 ($p < 0.01$) levels in GCF were found to be significantly lower in healthy subjects than in chronic periodontitis patients. Except CAL, SRP led to improvement in all examined clinical parameters, and also resulted in a significant decrease in the amounts of IL-1β, IL-8 and MMP-8 in comparison to baseline, especially 4 weeks after scaling and root planing ($p < 0.001$); however, the amounts of these humoral factors were still higher than those in control group. Author's observations specified a significant improvement in periodontal indices and marked decrease in GCF IL-8, IL-1β and MMP-8 levels after short-term nonsurgical therapy.

Fu YW et al. (2015)[20] conducted a study to evaluated whether periodontal treatment in patients with periodontitis and hyperlipidemia may have any effect on plasma lipids as well as proinflammatory cytokine levels. 109 patients with hyperlipidemia and chronic periodontitis were randomly divided into group 1 (n=55) underwent a standard cycle of supragingival mechanical scaling and polishing and group 2 underwent the adjunctive full-mouth intensive removal of subgingival dental plaque biofilms with the use of scaling and root planning. Periodontal parameters including Number of present teeth, BOP, PD, GML, CAL, as well as Biochemical parameters including triglyceride (TRG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density

lipoprotein cholesterol (HDLC), TNF-α, IL-1β, and IL-6 were evaluated before, 2 and 6 months after treatment. Two and 6 months after treatment, TRG levels were found significantly lower in group 2 than in group 1 (P<0.05), and the levels of HDL-C were significantly higher (P<0.05). Two and 6 months after therapy, the levels of TNF-α were significantly lower in group 2 than in group 1 (P<0.05), as were the levels of IL-1β (P<0.001) and IL-6 (P<0.001). This short-term, randomized clinical trial showed that intensive periodontal treatment improves serum lipid levels and decreases serum proinflammatory cytokine levels in patients with hyperlipidemia and chronic periodontitis.

Mahajani MJ et al. (2017)[21] assessed the influence of periodontal therapy on the crevicular fluid Interleukin-18 (IL-18) level in periodontal health and disease in Central Maharashtra population. Total 90 patients were included in the study and based on clinical attachment loss (CAL), probing pocket depth (PPD), gingival index (GI), and plaque index (PI) patients were divided into three groups: Group I with healthy patients, group II with chronic periodontitis, and group III with post-treatment patients having periodontitis (patients treated with scaling and root planing). GCF sample collection were done by microcapillary pipette and analyzed using enzyme-linked immunosorbent assay (ELISA) kits. The total amount of IL-18 in GCF was found to be significantly higher in group II when compared with groups I and III ($p < 0.05$). Also, Mean PI, PPD, CAL, and GCF volume were significantly higher in groups II and III compared with group I. The present study confirmed that the IL-18 level in GCF was lower in healthy patients, higher in periodontally involved patients, and reduced at baseline, 3 and 6 weeks after nonsurgical periodontal therapy. Thus, it can be concluded that, IL-18 might be potentially useful in distinguishing health from disease and observing periodontal disease activity.

Toker H et al. (2018)[22] evaluated the effect of interleukin-10 (-597) gene polymorphism and genotype distributions on chronic periodontitis (CP) development and IL-6 and IL-10 levels in GCF and serum before and after non-surgical periodontal treatment. A total of 105 participants were involved in this study and are equally divided into CP group consisted of 55 chronic periodontitis patients (CP group) and control group consisted of 50 healthy volunteers (HC group). Clinical parameters including Plaque index, gingival index, probing depth and clinical attachment level were recorded and GCF and blood samples were taken at both the baseline and the sixth week after non-surgical periodontal treatment. IL-6 and IL-10 levels in GCF and serum samples were determined via ELISA kits, IL-10 gene polymorphism was evaluated via PCR-RFLP by analyzing IL-10 gene at position -597. Periodontal treatment significantly improved clinical measurements in the CP group and significantly decreased GCF IL-6 and IL-10 levels. GCF IL-10 levels at Sixth week were significantly lower in patients carrying IL-10 AC+CC genotype compared to the patients carrying IL-10 AA genotype ($p<0.05$). Serum IL-6 and IL-10 levels were lower in patients carrying the IL-10 AA genotype compared to patients with IL-10 AC+CC genotype, but the difference was not significant ($p>0.05$). Thus, authors concluded that, IL-10 AA genotype carriers had lower IL-6 and IL-6/10 levels in serum; however, GCF IL-6/10 levels were similar in both genotypes. Within the limitations of study, a possible association between IL-10(-597) gene polymorphism and CP might be considered and IL-10 -597 AA genotype appeared to be a risk factor for chronic periodontitis. Furthermore, genetic polymorphisms in the IL-10 gene might be useful as a marker to diagnose susceptibility to CP.

Raj SC et al. (2018)[23] estimated and compared the interleukin-35 (IL-35) levels in GCF and serum among healthy, gingivitis, and chronic periodontitis (CP) individuals as well

as evaluated the effect of nonsurgical periodontal treatment (NSPT) on IL-35 level among patients with CP. A total of 60 participants were included in this comparative study and are divided into 3 groups Group I: healthy (n = 20), Group II: gingivitis (n = 20), and Group III: Chronic Periodontitis (n = 20), the subjects of Group III have been referred to as Group IIIA at baseline and IIIB for recording data 6 weeks after the NSPT. GCF samples collected from each individual at baseline and 6 weeks after NSPT for Group III individuals using color-coded 1–5-μL calibrated volumetric microcapillary pipettes. The serum was prepared from the blood sample and the GCF and serum samples were then assayed for IL-35 using enzyme-linked immunosorbent assay kit. It was found that the mean IL-35 concentration in GCF as well as serum was highest in Group IIIA and least in Group I; however concentration in GCF was found to be significantly higher ($P < 0.05$) for Group IIIA (70.26 ± 4.0 pg/ml), as compared to Group I (54.81 ± 22.3 pg/ml) and Group IIIB (55.72 ± 10.2 pg/ml). Individuals receiving NSPT exhibited a significant reduction in IL-35 levels as compared to CP individuals.

Mastromatteo-Alberga P et al. (2018)[24] in a prospective cohort study of 22 subjects determined the levels of IL-1α, IL-1β, TNF-α, IL-6, IL-6sR, IL-8, IL-10, MMP- 3 and MMP-8 in GCF, prior to and after NSPT in order to evaluate therapy response. The patients were equally divided into 2 groups (11 each): group 1- patients diagnosed with chronic periodontitis and group 2- healthy controls. Clinical measurements, including probing depth (PD), clinical attachment loss (CAL), gingival index (GI) and plaque index (PI) were evaluated. GCF samples were collected from one tooth per quadrant before and 45 days after NSPT using paper points. The levels of inflammatory mediators were measured by ELISA. Reduction in all clinical parameters were statistically significantly in both the groups after treatment. The concentration levels of

all cytokines and MMP-3 and MMP-8 in the GCF sample were higher in patients diagnosed with chronic periodontitis compared to the healthy group. All inflammatory mediators decreased after therapy, but did not reach control values; IL-6, IL-6sR, IL-10 and TNF-α, attained the highest reduction (70% - 54%); the vales of MMP-3, IL-8, IL-1α and IL-1β were reduced between 50% - 34%; and lowest decrease (28%) shown by MMP-8. After NSPT, all clinical parameters as well as cytokines levels were decreased. TNF-α, IL-6, IL-6sR, and IL-10 are the cytokines whose levels varied the most before and after NSPT, and could be used to evaluate therapy response.

Guruprasad CN et al. (2018)[25] conducted a study to estimate the levels of Interleukin-34 (IL-34) in GCF and plasma in periodontal health, disease and chronic generalized periodontitis-affected individuals after the nonsurgical periodontal treatment. A total of 30 individuals both males and females with age range 30–56 years were selected and divided into 3 groups based on the gingival index, probing pocket depth, clinical attachment level, and radiologic parameters (bone loss): Group I included 15 individuals with healthy periodontium, Group II included 15 individuals with chronic generalized periodontitis while Group II patients after 8 weeks of the treatment (scaling and root planning) constituted Group III. GCF samples and plasma samples were collected to estimate the levels of IL-34 using enzyme-linked immunosorbent assay kit. The mean concentration of IL-34 in GCF and plasma was found to be highest for Group II compared to Group I, and statistically significant decrease in IL-34 level after nonsurgical periodontal therapy in chronic generalized periodontitis group ($P < 0.05$). Thus authors concluded that, IL-34 can be considered as an "inflammatory marker" of periodontal disease and can be explored in the future as a potential therapeutic target in the treatment of periodontal disease.

Zekeridou A et al. (2019)[26] in a study compared cytokine levels in addition to assess the levels in peripheral blood with those in the GCF and evaluated the impact of NSPT on the prevalence of high levels of 12 biomarkers in serum. Twenty-four patients with chronic periodontitis were assigned to Group P and sixty periodontally healthy individuals were assigned to Group H. All diseased sites were treated with deep scaling and root planning under local anesthesia. Serum and GCF samples were obtained at baseline (BL) and 1 and 3 months after periodontal treatment (M1 and M3). Using the Bio-Plex bead array multianalyte detection system samples were assessed for 12 cytokines (IL-1β, IL-1 receptor antagonist (IL-1ra), IL-6, IL-7, IL-8, IL-17, basic fibroblast growth factor (b-FGF), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), interferon γ (IFN-γ), macrophage inflammatory protein 1β (MIP-1β), TNF-α, and VEGF). In the periodontitis group there were significant correlations between GCF and serum values for IL-1ra, IL-6, and INF-γ at BL and for macrophage inflammatory protein 1β at M3 after treatment. For INF-γ, highest incidence of peaks at BL was found (37.5% of the periodontitis subjects). For the four biomarkers with a detection frequency of >75% at BL (IL-1ra, IL-8, macrophage inflammatory protein 1β, and vascular endothelial growth factor), there were no significant difference was observed for the P group over time or between the two groups at BL. For certain cytokines, significant correlation was found between the GCF and serum and the fact that periodontitis subjects exhibited high peaks for several inflammatory markers in serum may support the hypothesis that the inflammatory reaction due to periodontitis is not restricted to the diseased sites, also periodontal therapy did not seem to have any significant impact on the systemic cytokine levels.

2. Studies on effect of PDT on cytokines

Giannopoulou C et al. (2012)[27] performed a randomized, split-mouth clinical trial to compare the local biologic effects of photodynamic therapy (PDT), diode soft laser (DSL) therapy, and conventional deep scaling and root planing (SRP) in residual pockets. Based on a history of previous treatment for periodontitis and the persistence of sites with probing depths >4mm and bleeding on probing, 32 systemically healthy individuals including 23 males and 9 females, with age range of 36 to 74 years were recruited. Residual pockets were debrided with an ultrasonic device and then randomly assigned either to PDT, DSL, or SRP. GCF samples were collected before treatment (baseline) and at 2 weeks, 2 months, and 6 months after treatment by means of a membrane strip. Levels of 13 cytokines (IL-1 β, IL-1ra, IL-8, IL-17, basic fibroblast growth factor (b-FGF), granulocyte colony-stimulating factor (GCSF), GM-CSF, IFN-γ, MCP-1, macrophage inflammatory protein 1a (MIP-1a), MIP-1b, TNF- α, and VEGF) and nine acute-phase proteins (a-2 macroglobulin (a2M), CRP, haptoglobin, and serum amyloid P, ferritin, fibrinogen, procalcitonin, serum amyloid A, and tissue plasminogen activator) were measured using a bead-based multiplexing analysis system. Compared with baseline, levels of interleukin-17, b-FGF, GCSF, GM-CSF, and MIP-1a were significantly lower in 14 days and 2 months after treatment with PDT, DSL, or SRP. At 6 months, the levels of IL-17, b-FGF, GM-CSF, and MIP-1a were still significantly reduced compared with baseline. The levels of IFN-γ and TNF-α decreased significantly 2 months after treatment, but TNF- α increased from baseline values 6 months after treatment. Among three treatment modalities, there were no significant differences for any biochemical parameter at any time point were observed. Regardless of treatment modality levels of several cytokines and acute-phase proteins were significantly changed. DSL and PDT achieved changes in cytokine and acute-phase

protein levels comparable to traditional SRP therapy, avoiding any mechanical surface interaction and damage.

Luchesi VH et al. (2013)[28] conducted a study to investigate the effect of photodynamic therapy (PDT) as an adjunct to mechanical therapy in furcations. Total 37 subjects were included in this double-blind, parallel, randomized controlled clinical trial which was conducted in subjects presenting class II furcations. The subjects were randomly allocated to a test group (n = 16) treated with PDT; or control group (n = 21) treated with non-activated laser/ only photosensitizer. Clinical, microbiological and cytokine pattern evaluation was performed at baseline, 3 and 6 months. GCF was collected from furcation sites at baseline and at 3 and 6 month re-evaluations by filter paper strips. Cytokine levels [granulocytemacrophage colony-stimulating factor (GM-CSF), interferon (IFN)-c, interleukin (IL)-10, IL-12, IL-13, IL- 1b,IL-4, IL-6, IL-8 and tumour necrosis factor (TNF)-α] in GCF were determined using the high sensitivity human cytokine 10-plex. At 3 months, inter-group analyses showed statistically significant reduction in GM-CSF, IL-8, IL-1β and IL-6 levels only in the PDT group ($p < 0.05$). At 6 months, lower IL-1β levels were also observed in the PDT group ($p < 0.05$) and IL-4 and IL-10 levels were increased in both groups. Hence, it was concluded that, PDT did not validate clinical benefits for class II furcations; though, advantages in a reduction in periodontopathogens and local levels of cytokines were demonstrated.

Pourabbas R et al. (2014)[29] in vivo study evaluated the post-treatment clinical parameters and GCF cytokine profiles in patients with moderate to severe chronic periodontitis (CP), treated by either scaling and root planing (SRP) alone or SRP in addition to photodynamic therapy (PDT). Twenty-four pairs of contralateral maxillary

or mandibular teeth affected with moderate-to-severe CP were selected. After SRP, selected teeth were randomized to receive either no further treatment or a single application of PDT using a 638-nm laser and toluidine blue. Clinical parameters including probing depth, bleeding on probing, clinical attachment level, gingival recession and biochemical parameters which includes interleukin-1β, tumor necrosis factor (TNF)-α, and matrix metalloproteinase 8 and 9 were also evaluated at baseline and 3 months post-intervention. The paper strips were used for GCF sampling and ELISA were used for cytokine analyses. To determine the total levels of oral PMNs, an oral rinse assay was also performed before and 3 months after the treatments. Only TNF- α was found to be significantly improved in the PDT + SRP versus SRP group. Within each group, significant improvements ($P < 0.001$) were found for all variables in 3-month follow-up compared with baseline. Total levels of PMNs were reduced for all patients compared with baseline levels ($P < 0.001$). Thus, Authors concluded that, in patients with CP, a single application of PDT (using a 638-nm laser and toluidine blue) did not provide any additional benefit to SRP in terms of clinical parameters or inflammatory markers 3 months following the intervention.

Kolbe MF et al. (2014)[30] in a randomized controlled trial assessed the clinical, microbiologic, immunoinflammatory and patient-centered effects of photodynamic therapy (PDT) as monotherapy during periodontal maintenance. 22 patients with chronic periodontitis (CP) presenting at least three residual pockets (probing depth [PD] ≥5 mm with bleeding on probing [BOP]) were selected for this split-mouth study. The selected sites randomly received the following: 1) PDT; 2) photosensitizer (PS); or 3) scaling and root planing (SRP). Clinical, microbiologic (real-time polymerase chain reaction analyses), cytokine pattern (multiplexed bead immunoassay), and patient-

centered (regarding morbidity) evaluations were performed at baseline and 3 and 6 months. Only patients in the PDT protocol exhibited increased levels of anti-inflammatory cytokine IL-4 and decreased levels of proinflammatory cytokine IL-1β and IL-6 throughout the study (P <0.05). Intergroup analyses showed reduced IL-10 levels and increased interferon-γ and IL-1β levels in the PS protocol when compared with the other therapies during follow-ups (P <0.05). All therapies promoted similar improvements in clinical parameters throughout the study (P <0.05), except that BOP was not reduced in the PS protocol (P >0.05). No differences in morbidity were observed between the therapies (P >0.05), although the need for anesthesia was higher in SRP-treated sites (P <0.05). Thus, PDT as monotherapy exhibits advantages in cytokine modulation and may be used as an alternative and non-invasive therapeutic approach to treat residual pockets. It could represent a new therapeutic approach in the supportive periodontal maintenance routine.

Mistry A et al. (2016)[31] evaluated the efficacy of combination therapy of diode laser and photodynamic therapy (PDT) as an adjunct to scaling and root planing (SRP) on interleukin-17 (IL-17) levels in GCF in chronic periodontitis. 30 Indian adults (19 males and 11 females with a mean age of 38.03 years) with chronic periodontitis were recruited and randomly allocated to the test group (SRP plus combined therapy using diode laser and PDT) or control group (SRP alone). Microcapillary pipette was used for collection of GCF and IL-17 was analyzed using an ELISA kit at baseline and 3 months. A significant decrease in GCF levels of IL-17 was observed in both treatment groups 3 months after treatment ($P < 0.001$). No significant difference was found among treatment groups ($P > 0.05$). The results of the study showed that, both treatment modalities resulted in significant decrease in GCF IL-17 level in patients with chronic

periodontitis suggesting the efficacy of combination therapy of diode laser and PDT as an adjunct to SRP on reducing levels of GCF IL-17 in patients with chronic periodontitis.

Teymouri F et al. (2016)[32] designed a study to evaluate and compare the impact of laser and photodynamic therapy as complementary periodontal treatments on the clinical status and levels of inflammatory mediators as new mechanisms for evaluating periodontal diseases, especially the new mediators. Three quadrants in 12 patients with chronic periodontitis aged 30-60 years were selected in this clinical trial. After the first phase of periodontal treatment, one of the three quadrants was determined as the control group, one was treated by diode laser, and one underwent photodynamic therapy. The clinical parameters were recorded and GCF samples were collected by paper points no.25 at baseline, 2 and 6 weeks later. GCF samples were analyzed by special ELISA kit. The data were statistically analyzed by using Friedman, ANOVA, and LSD post-test resulted in significant reduction in the levels of IL-1β, IL-17, clinical attachment loss, and pocket depth in the three treatment groups ($p< 0.000$). At the baseline, up to 2 weeks, and 2-6 weeks, three treatment modalities significantly reduced the IL-1β and IL-17 levels ($p< 0.05$). Diode laser and photodynamic therapy significantly decreased the average bleeding on probing over time ($p< 0.000$ and $p< 0.002$, respectively). Within the conditions of this study, it can be concluded that regarding the anti-inflammatory effects of periodontal treatment methods, adjunctive PDT and laser treatment significantly reduce the level of inflammatory mediators and also improve the clinical symptoms, particularly BoP, which is again more efficient when prolonged.

de Melo Soares, MS et al. (2019)[33] performed a split-mouth, double-masked, randomized, controlled clinical trial to investigate the additional influence of multiple applications of antimicrobial photodynamic therapy (aPDT) in smokers with chronic periodontitis. Twenty-two patients (6 males and 16 females, aged 39 to 61 years) were enrolled and the upper quadrants in each patient were allocated randomly to one of two treatment protocols: SRP + aPDT or SRP alone. aPDT was performed by using a laser light source of 660 nm wavelength through a photosensitizer with repeated application after 2, 7, and 14 days. Plaque index, probing depth, clinical attachment level, and bleeding on probing were assessed at baseline, 30, and 90 days after the SRP. GCF and subgingival plaque samples were collected for immunological and microbiological analysis, respectively. Levels of anti-inflammatory cytokines and bacterial species were found to be comparable in both groups at day 90 after treatment. aPDT as an adjunct to SRP did not demonstrate statistically significant improvement in clinical parameters when compared with SRP alone. Thus, multiple episodes of aPDT adjunctive to non-surgical treatment did not improve significantly the clinical, immunological, and microbiological parameters in smokers when compared with SRP alone in patients with chronic periodontitis.

3. Studies on influence of NSPT and PDT (adjunctive therapy) on chronic periodontitis

Christodoulides N et al. (2008)[14] evaluated the clinical and microbiological effects of the adjunctive use of PDT to non-surgical periodontal treatment. 24 patients with chronic periodontitis were recruited and were randomly divided into 2 groups: test group treated with scaling and root planing followed by a single episode of PDT and

Control group treated with SRP using hand and sonic instruments. FMPS, FMBS, PD, GR, and CAL were measured at baseline and at 3 and 6 months post therapy. Microbiologic evaluation of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Treponema denticola, Parvimonas micra, Fusobacterium nucleatum, Campylobacter rectus, Eubacterium nodatum, Eikenella corrodens, and Capnocytophaga spp. was performed by using a commercially available polymerase chain reaction test at baseline, 3 and 6 months following therapy. A statistically significantly greater improvement in FMBS was found in the test group, at 3 and 6 months. However, with respect to CAL, PD, FMPS, or microbiologic changes there were no statistically significant differences between the groups were found. The authors concluded that the additional application of a single episode of PDT to SRP resulted in a significantly higher reduction in bleeding scores compared to SRP alone. However failed to achieve an additional improvement in terms of PD reduction and CAL gain.

Campos GA et al. (2012)[34] performed a randomized controlled clinical trial where they evaluated the effect of a single photodynamic therapy (PDT) as an adjunct to scaling and root planning (SRP) in residual pockets in single-rooted teeth. 15 systemically healthy subjects presenting at least two residual pockets (probing pocket depth (PPD) ≥5 mm with bleeding on probing (BoP)) in single root teeth in supportive periodontal therapy were recruited. The selected sites were then allocated to PDT + SRP or SRP. In sites treated by PDT as an adjunctive to SRP, the laser system included a handheld battery-operated diode laser having wavelength of 660 nm with a power output of 60 mW, and energy density of 129 J/cm2, along with methylene blue as a photosensitizer (10 mg/ml). Clinical parameters were evaluated at baseline and 3 months after therapy

which included position of the gingival margin (PGM), relative CAL (RCAL), Probing pocket depth (PPD), bleeding on probing (BoP), full mouth plaque score (FMPS) and bleeding score (FMBS). Clinical parameters improved significantly in both the groups ($p< 0.05$), whereas higher PPD reduction and CAL gain were observed in the PDT + SRP group at 3 months ($p<0.05$). In addition, sites treated by the combined approach yielded a significant reduction in the number of sites with PPD <5 mm without BoP after 3 months compared to sites treated by conventional SRP alone ($p< 0.05$). The study concluded that PDT as an adjunctive to mechanical debridement demonstrated additional clinical benefits for residual pockets in single-rooted teeth and may be an alternative therapeutic strategy in supportive periodontal maintenance.

Theodoro LH et al. (2012)[35] in a split mouth study investigated the long-term clinical and microbiological effects of photodynamic therapy (PDT) associated with nonsurgical periodontal treatment in 33 chronic periodontitis patients. In each patient three sites were randomly allocated to 3 groups: (1) SRP group (2) SRP and irrigation with TBO group and (3) SRP, irrigation with TBO and low level laser irradiation (PDT group). Clinical parameters including visible plaque index, bleeding gingival index, BOP, PD, GR and CAL were evaluated at baseline and after 60, 90 and 180 days. Additionally, subgingival plaque samples were collected for microbiological analysis of A. actinomycetemcomitans (Aa), P. gingivalis (Pg), P. intermedia (Pi), Tannerella forsythia (Tf) and Prevotella nigrescens (Pn) by PCR. Inter- and intragroup comparisons were performed for all variables at the different evaluation time points. All treatment groups showed a significant reduction in the proportion of sites positive for periodontopathogens and an improvement in all clinical parameters, and at 60, 90 and 180 days compared to baseline ($p<0.05$). None of the periodontal parameters showed a

significant difference among the groups ($p>0.05$). Compared to SRP alone, PDT treatment led to a significant reduction in the percentage of sites positive for all bacteria ($p<0.05$) at 180 days. This study suggested that PDT as an adjunct to periodontal treatment produced statistically significant reductions in some of the key periodontal pathogens but produced no significant benefit in terms of clinical outcome.

Betsy J et al. (2014)[36] assessed the potential of antimicrobial photodynamic therapy (aPDT) as an adjunct to scaling and root planing (SRP) in the treatment of chronic periodontitis. 90 patients (51females and 39 males) with untreated chronic periodontitis were recruited and randomly divided into 2 groups: Test group- (SRP with aPDT) and Control group- (SRP alone). Clinical parameters recorded were probing pocket depth (PPD) clinical attachment levels (CAL), gingival index (GI), gingival bleeding index (GBI) and plaque index (PI) before and after treatment. Halitosis was recorded by patient's hand on mouth technique at baseline, 1 month, 3 months and 6 months of treatment. Intergroup and intra-group statistical analyses were performed. Statistically significant reduction showed by PPD and CAL in the test group at 3 months and 6 months as compared to the control group ($p<0.05$). A statistically significant improvement in GI and GBI was seen for the test group after 2 weeks and 1 month of aPDT ($p<0.01$), whereas the improvement in GI and GBI at 3 months and in PI at 2 weeks after aPDT was less ($p<0.05$). Also, a significant difference was detected for the test group at 1 month in terms of halitosis ($p<0.05$), which did not persist for long. This study concluded that aPDT acts as a beneficial adjunct to SRP in nonsurgical treatment and management of chronic periodontitis in short-term.

Alwaeli HA et al. (2015)[12] compared the effect of adjunctive antimicrobial photodynamic therapy (aPDT) plus scaling and root planing (SRP) with SRP alone, which can last for 1 year. Total 136 sites in 16 patients with previously untreated chronic periodontitis, at least one premolar and one molar in every quadrant (minimum, four teeth/quadrant) and at least one tooth with attachment loss of ≥4 mm in every quadrant were included in this randomized clinical trial. In all patients, two randomly assigned quadrants were treated with SRP and the other two were treated with SRP + aPDT. The clinical parameters of probing pocket depth (PPD), bleeding on probing (BOP), and clinical attachment level (CAL) were evaluated at baseline and after 3, 6, and 12 months. The Mann–Whitney test was used for comparisons between the treatment groups and showed no significant differences between the groups at baseline. PPD and BOP showed significant reduction, and CAL showed significant gain from baseline for all three time points in both groups. In addition, there were significantly greater reduction and gain for SRP + aPDT than for SRP at all three time points. In this study, no adverse effects of aPDT were observed, demonstrated significant improvement in all evaluated clinical parameters for at least 1 year and suggested that aPDT as an adjunctive therapy to SRP represents a promising therapeutic concept for persistent periodontitis.

Birang R et al. (2015)[37] compared the impact of adjunctive laser therapy (LT) and photodynamic therapy (PDT) on patients with chronic periodontitis. Twenty patients with at least three quadrants involved and presenting 4-8 mm deep pockets were included in split-mouth, double blind, randomized controlled clinical trial. Periodontal treatment comprising scaling and root planning (SRP) was performed for the whole mouth and divided into 3 groups: Group A (treated with SRP alone), Group B (SRP

with LT) Group C (SRP with PDT). The clinical indices PPD (periodontal pocket depth), CAL (clinical attachment level), Plaque Index (PI), Papillary Bleeding Index (PBI) were measured at baseline 6 weeks and 3 months after treatment. The microbiological sample analysis was performed with real time polymerase chain reaction method (RT-PCR) to evaluate the amount of Actinobacillus actinomy-cetemcomitans, Porphyromonas gingivalis and Treponema denticola. All groups showed statistically significant improvements in terms of CAL gain, PPD reduction, PBI and microbial count compared to baseline ($P < .05$). The results showed more significant improvement in the 6-week evaluation in terms of CAL in groups B and C than in group A ($P < .05$). Group B also revealed a greater reduction in PPD than the other treatment modalities ($P < .05$). All bacterial species decreased significantly from baseline to the 3-month evaluations ($P < .05$) with no significant difference between the three treatment modalities. Thus, obtained data suggested that adjunctive LT and PDT have significant short-term benefits in the treatment of chronic periodontitis. Furthermore, LT showed minimal additional advantages compared to PDT.

Sreedhar A et al. (2015)[38] in a clinical and microbiologic study compared the efficacy of photodynamic therapy (PDT) using curcumin as photosensitizer and curcumin as a gel to be used as an adjunct to scaling and root planing (SRP) with SRP alone in the treatment of chronic periodontitis. 60 sites from fifteen untreated CP patients were randomly assigned for one of the treatment modalities; 1) SRP alone, (2) SRP + Curcumin application for 5 min, (3) SRP + Curcumin application for 5 min + irradiation with blue light emitting diode of wavelength 470 nm for 5 min. (Curcumin PDT) on "0" day. (4) SRP + Curcumin PDT on 0, 7th and 21st day. The clinical parameters included plaque index (PI), bleeding on probing (BOP) measured by sulcus bleeding index (SBI),

probing pocket depth (PPD), clinical attachment level (CAL) measured using a UNC-15 probe at the baseline & at 3rd month. The site with PPD was selected from each quadrant for bacterial sampling as well as culturing for Aa and other black pigment producing microorganisms (BPB) like Pg & Pi. Intergroup comparison showed highly significant reduction in clinical parameters such as PI, SBI, PPD, and CAL ($P < 0.000$). Statistically highly significant improvement was observed suggested that single application of PDT with curcumin as a PS gives better results than curcumin alone after SRP.

Decker EM et al. (2016)[39] compared the antibacterial effect of 10 different mouthrinse formulations with that of conventional and modified PDTplus on the one hand, and on the other hand, the antibacterial effect of the mouthrinse applications combined with conventional PDT as well as combined with newly modified PDTplus on six representative pathogens associated with periodontitis and peri-implantitis. Six representative periodontitis-associated bacterial strains [Aggregatibacter actinomycetemcomitans (Aa, ATCC 43718); Actinomyces viscosus (Av, DSMZ 43798); Fusobacterium nucleatum (Fn, ATCC 10953); Porphyromonas gingivalis (Pg, ATCC 33277); Veillonella atypica (Va, ATCC 17744); and Streptococcus gordonii (Sg, ATCC 33399; DSMZ)] were grown for 24 h at 37°C anaerobically. After mixing the individual cell pellets they were exposed to 10 different antiseptic mouthrinse formulations: chlorhexidine (0.2%, 0.06%, CHX); CHX + cetylpyridinium chloride (each 0.05%); sodium hypochlorite (0.05%); polyhexanide (0.04%, PHMB1; 0.1%, PHMB2); octenidine dihydrochloride (0.1%); fluoride (250 ppm); essential oils; povidone iodine (10%); and saline (0.9%, NaCl) as control. Furthermore, the bacterias were treated with conventional PDT and a new modified photodisinfection combining

photosensitizer with hydrogen peroxide to PDTplus. A combined application of antiseptic exposure was followed by use of PDT or PDTplus. The microbial viability was characterized by evaluating colony growth and fluorescence-based vitality proportions. Complete elimination of bacterial growth was reported with CHX (0.2%), CHX/cetylpyridinium chloride and octenidine dihydrochloride. Moderate reduction of colony growth was found with conventional PDT while the modified PDTplus achieved maximum antimicrobial effect. Thus, Authors concluded that a combination therapy of preceding chemotherapeutical exposure and subsequent photodisinfection may be a more effective and promising antibacterial treatment than single applications of the antiseptic methods. The modified PDTplus using oxygen-enriched toluidine showed a superior antibacterial effect on periodontal pathogens to conventional PDT and to the majority of the investigated mouthrinses.

Ravi KR et al. (2016)[40] performed a clinico-microbiological study where they investigated the efficacy of adjunctive use of PDT with scaling and root planing as compared with SRP alone in the treatment of 20 chronic periodontitis patients. Patients having probing pocket depths (PDs) of ≥5 mm were randomly divided into control group and test group with ten patients in each group. Full-mouth SRP was performed in both the groups, followed by application of PDT in test group. Plaque index (PI), Gingival index (GI), Probing depth (PD), and Clinical attachment level (CAL) was assessed at baseline and after 3 months. Microbiological assessment of P gingivalis, T forsythia, and T denticola was done by PCR at baseline and 3 months post therapy. On assessment of the clinical and microbiological parameters statistically significant reduction was found in all the parameters in test group as compared to control group. They concluded that additional improvement in periodontal parameters shown by PDT

in conjunction with SRP when compared to SRP alone also has a beneficial effect in chronic periodontitis patients.

Tabenski L et al. (2016)[41] explored the additional influence of either antimicrobial photodynamic therapy (aPDT) or local application of minocycline microspheres (MC) on clinical and microbiological healing results in deep periodontal pockets having PPD ≥6 mm following SRP. Forty-five patients with chronic periodontitis were evaluated in this randomized clinical trial and were equally divided into 3 groups: test group (aPDT + SRP), positive control group (MC + SRP), and negative control group (SRP-alone). Clinical parameters Approximal plaque index (API), PBI, BOP, PPD, and CAL as well as microbiological healing parameters were recorded in every patient for four experimental teeth at baseline, 6 weeks, and 3, 6, and 12 months. Wilcoxon signed-rank test and Mann-Whitney U test showed significant improvements in clinical and microbiological parameters for all groups after 6 weeks and 3, 6, and 12 months ($\alpha = 0.05$). But, Differences between groups were not statistically significant. However, all three treatment modalities achieved statistically significant improvements in clinical healing parameters during the 12-month examination period. Statistically significant reductions of Porphyromonas gingivalis (Pg), Tannerella forsythia (Tf), and Treponema denticola (Td) were observed in each group for most follow-ups during the 12-month period. Within the limitations of this study, it was concluded that neither the applied aPDT system nor MC showed a significant additional effect on clinical and microbiological healing outcomes in deep periodontal pockets compared to SRP alone.

Shingnapurkar SH et al. (2017)[42] assessed the effect of adjunctive photodynamic therapy (PDT) (using 810 nm diode laser and Indocyanine green as photosensitizer) in

chronic periodontitis (CP). Patients with untreated CP with probing pocket depth (PPD) >5 mm were included and randomly divided into 2 groups: Group A – Treated with scaling and root planing (SRP) only (control site) and Group B– Treated with SRP + PDT (test site). Each group had thirty sites. Plaque Index (PI), Gingival Index (GI), Probing Pocket Depth (PPD) and Relative Attachment Level (RAL) were evaluated at baseline, 1 month and 3 months and statistical significant differences were found in PPD and RAL, 3 months after treatment in test group as compared to the control group. This study suggested that clinical outcomes of conventional SRP can be improved by adjunctive PDT in patients with chronic periodontitis.

Suchetha A et al. (2017)[43] compared the efficacy of PDT with scaling and root planing (SRP) and also to compare the efficacy of two different concentrations of photosensitizer (methylene blue 0.005% and 0.01%) in the treatment of chronic periodontitis. 45 patients affected with moderate-to-severe chronic periodontitis were included in the study and were divided into three groups. Group I (n = 15): treated with SRP only, Group II (n = 15): treated with SRP + PDT (0.005% methylene blue), Group III (n = 15): treated with SRP + PDT (0.01% methylene blue). The clinical parameters, plaque index (PI), gingival index (GI), and probing pocket depth were recorded at baseline, 1 month, and 3 months using UNC-15 Probe. After SRP, PDT was performed using methylene blue dye (0.005% and 0.01%) and diode laser with 665 nm wavelength for 60 s. there were no statistically significant differences seen between the groups with respect to reduction in PI, GI, and probing pocket depth (P > 0.05). This study concluded that the additional application of a single episode of PDT to SRP failed to result in an additional improvement in terms of reduction in plaque score, GI score, and pocket probing depth.

Andersen RC et al. (2017)[44] in a randomized, examiner-blinded, multicenter parallel-group study of 121 patients assessed the efficacy and safety of a antimicrobial photodynamic therapy (aPDT) in the treatment of adult patients diagnosed with chronic periodontitis (CP), by evaluating gain in clinical attachment level (CAL), reduction in probing depth (PD), and reduction in bleeding on probing (BOP). Following SRP the patients were randomized into 2 groups: Control group, receiving SRP alone and Test group, receiving SRP followed by aPDT. aPDT was carried out using a diode laser of 670nm and 0.2 W in power. For bacterial staining a methylene blue based photosensitizer was used. Results of this study when assessed by average CAL gain (0.19mm, $p<0.0001$) or PD reduction (0.15mm, $p<0.0001$) showed that patients receiving adjunctive aPDT with methylene blue experienced significantly better outcomes than SRP alone. These results were apparent at 6-weeks as well as the 12-week follow-up period. This outcome demonstrated the safety and efficacy of aPDT therapy in the nonsurgical treatment of chronic adult periodontitis.

AlAhmari F et al. (2019)[45] compared the efficacy of scaling and root planing (SRP) with and without adjunct antimicrobial photodynamic therapy (aPDT) in the treatment of chronic periodontitis (CP) among cigarette-smokers and never-smokers. In total, 83 male patients with CP (Group-1: 42 cigarette-smokers and; Group-2: 41 never smokers) were included in this 12-week parallel-arm, randomized controlled clinical trial. Treatment wise, these individuals were divided into two subgroups as follows: (a) SRP alone and (b) SRP with adjunct aPDT. Demographic information was collected using a questionnaire. Periodontal parameters plaque index [PI], bleeding on probing [BOP], clinical attachment loss [AL] and probing pocket depth ≥ 4mm [PD] were measured at baseline and at 1 month and 3 months' follow-up. The Kruskall-Wallis test was used to compare the changes in periodontal inflammatory parameters and the changes between

treatment modalities. At 1 month and 3 months' follow-up, PI ($P < 0.05$), PD ($P < 0.05$) and clinical AL ($P < 0.05$) were higher among all individuals in group-1 compared with group-2. In Group-2, PI, BOP, PD and clinical AL were comparable among all individuals at 1 month and 3 months' followup. The authors concluded that outcomes of SRP with or without aPDT for the treatment of CP are compromised in cigarette smokers. Among never-smokers with CP, outcomes of SRP with or without aPDT are comparable. However, the significance of aPDT in this regard remains questionable.

Hill G et al. (2019)[46] in a prospective clinical study evaluated the efficiency of indocyanine green (ICG)-based adjunctive antimicrobial photodynamic therapy (aPDT) with non-surgical treatment of 20 chronic periodontitis patients. Affected teeth of 20 patients were treated with scaling and root planing (control group). Using a split mouth design, two quadrants received additional ICG-based (perio green®, 0.1 mg/ml) aPDT (test group) with a diode laser at 808 nm (100 mW at 2 kHz). The parameters bleeding on probing (BOP), sulcus fluid flow rate (SFFR) and microbial analysis were clinically assessed at baseline (BL), two weeks (U1), three months (U2) and six months (U3) after treatment. The relative attachment level (RAL) with probing depths (PD) and gingival recession (GR) were also measured at baseline, and three months and six months after treatment. Microbial analysis of Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Tannerella forsythia (Tf) and Treponema denticola (Td) determined by real-time chain reaction (RTD-PCR). Median values for BOP, RAL, PD, decreased significantly in both groups after three months of treatment ($p \leq 0.05$) without significant difference between the groups. Two weeks after treatment, the SFFR showed significantly lower mean values in the test group (aPDT). Following aPDT, significant reduction was found in Pi and Td compared

to control group. Thus authors concluded that, with the exception of SFFR, the adjunctive treatment could not improve relevant clinical parameters under the applied treatment modalities. ICG-based aPDT treatment did not cause adverse side effects. Consequently, further research to identify the precise modes of action of ICG and further clinical prospective trials are obligatory.

4. Studies on Interrelationship between IL-6, IL-8, IL-10 and chronic periodontitis

Babel N et al. (2006)[47] analyzed the -1082 interleukin-10 (IL-10), -308 tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta 1 (TGF-β1) (codons 10 and 25), -174IL-6, and +874 interferon-gamma (IFN-γ) gene single nucleotide polymorphisms in a cohort of patients with chronic periodontitis (CP). A total of 236 patients were enrolled in this study, out of which a total of 122 consecutive patients with CP were included in the CP group while the control group consisted of 114 unrelated, ethnically matched white individuals with no signs of periodontitis. TNF- α, TGF-b, IFN- γ, IL-10, and IL-6 gene SNPs were analysed by the polymerase chain reaction–sequence-specific primer (PCR-SSP) using a commercially available cytokine genotyping primer pack. The number of individuals carrying the -174IL-6 CC genotype was significantly higher in periodontitis patients than in the control group (odds ratio [OR] = 1.896; 95% confidence interval [CI] = 1.106 to 3.250; P = 0.0283). The TGFb1 (codon 25) GG (Arg25/Arg25) genotype was detected more frequently in control subjects than in periodontitis patients (OR = 0.459; 95% CI = 0.230 to 0.920; P = 0.0421). Thus, it was concluded that, the -174IL-6 and TGF-b1 (codon 25) single nucleotide polymorphisms are associated with susceptibility to chronic periodontitis in the population studied.

Rescala B et al. (2010)[48] conducted a cross-sectional study to determine the GCF levels of IL-1β, IL-2, IL-4, IL-8, and IFN-γ and elastase activity between subjects with generalized chronic periodontitis (GCP) and generalized aggressive periodontitis (GAgP), and to compare their levels to subjects with only gingivitis. Clinical data were obtained from 37 subjects and were divided into 3 groups: GCP (n=20), GAgP (n=17), and gingivitis (n=10). Paper strips were used for collection of GCF samples and the levels of IL-1b, IL-2, IL-4, IL-8, and IFN- γ were measured using a multiplexed bead immunoassay. Elastase activity was assessed by an enzymatic assay. Analysis of Subgingival plaque samples were done using checkerboard DNA-DNA hybridization. Significance of differences among groups for immunologic and microbiologic data was examined using Kruskal-Wallis adjusting for multiple comparisons and in levels of GCF biomarkers and subgingival bacterial species there were no statistically significant differences were found between subjects with GCP and GAgP. Mean clinical parameters and GCF volumes were found to be higher in patients with GCP and GAgP compared to the gingivitis group. Higher levels of IL-1β and higher elastase activity were found in deep sites compared to shallow sites in both periodontitis groups ($P < 0.05$). Significantly higher levels of the red complex species were found in patients with GCP and GAgP than gingivitis ($P < 0.05$). Findings of the study showed no statistically significant differences in the measured immunologic and microbiologic parameters between subjects with GCP and GAgP.

Noh et al. (2013)[49] quantified IL-6, IL-8 and TNF-α levels in the human gingival tissues of patients with periodontitis and assessed the correlation of these three cytokines with each other. Human gingival tissues from 19 patients with periodontitis

were collected whilst the patients underwent periodontal surgery. Then the tissues were homogenized, centrifuged and the protein in the supernatant was quantified. The assessment of the IL-6, IL-8 and TNF-α levels in the tissues was performed by ELISA, and the mean levels of IL-8 were found to be higher than those of the other two cytokines. The expression of IL-6 and IL-8 showed a positive correlation (r=0.932, P=0.01), whereas TNF-α levels were not correlated with IL-6 or IL-8 levels. The results suggested that IL-6, IL-8 and TNF-α may be relevant in the pathophysiology of periodontitis, and the measurement of these cytokines may be beneficial in the identification of patients with periodontitis.

Lagdive SS et al. (2013)[10] correlated the levels of IL-8 with the clinical parameters of chronic periodontitis. Based on Radiographic examination and clinical periodontal assessments 80 subjects were divided into experimental (mild 20, moderate 20, severe 20) and control (20) groups. GCF was collected from patients with adult periodontitis and clinically healthy gingiva for 30 s using a Periopaper strip and the volume of the sample determined. Following elution of the fluid, assays for IL-8 were carried out by enzyme-linked immunosorbent assay (ELISA). Experimental group demonstrated significantly higher levels of IL-8 as compared to the control group ($P < 0.01$). The clinical parameters were found positively correlated to IL-8, suggesting that the GCF IL-8 exhibited dynamic changes upon severity of periodontal disease ($P < 0.05$). This data suggested that level of IL-8 is associated with periodontal status. The level of IL-8 in GCF is valuable in detecting the inflammation of periodontal tissue.

Fenol A et al. (2014)[50] evaluated the levels of IL-10 in GCF in healthy controls, subjects with gingivitis and those with periodontitis and compared its levels in the three

study groups. Based on GI (Gingival Index), OHI-S (Oral Hygiene Index-Simplified), PPD (Probing pocket depth) and CAL (Clinical attachment level), 91 subjects were divided into three groups: healthy, gingivitis and periodontitis. Collection of GCF samples were done using micropipette and IL-10 levels were analyzed using ELISA. Amongst 3 groups the highest mean concentration of IL-10 was obtained for gingivitis group (1128.19 ± 532.90 mg/ml), lowest for control group (648.96 ± 505.75) and for periodontitis (956.22 ± 475.49) group the mean IL-10 level was found to be intermediate between the other two groups. There was statistically significant difference found in IL-10 levels between control and gingivitis group ($p<0.05$) and control and periodontitis group ($p<0.05$). But difference in IL-10 levels between gingivitis and periodontitis group did not reach the level of statistical significance ($p>0.05$). This data suggested the protective role of anti-inflammatory cytokine, IL-10 in limiting progression of gingivitis to periodontitis.

Longo PL et al. (2015)[51] conducted a study to explore whether Type 2 diabetes mellitus (T2DM) and glycemic control interfere in inflammatory markers profiles in gingival crevicular fluid (GCF) in periodontitis patients. For this, the study population was divided into 3 groups; seven with adequate glycemic control (glycated hemoglobin [HbA1c] <8.0%) (DMA + P) and seven with inadequate control (HbA1c ≥8.0%) (DMI + P). Seven chronic periodontitis patients without diabetes formed the control group (P). Standard paper Strips was used for collection of GCF and cytokines levels were determined using multiplex beads immunoassay. Kruskal-Wallis and Dunn's multiple comparison tests showed no statistical differences between levels of interleukin-6 (IL-6), IL-8 and tumor necrosis factor-α. Cytokines profile of GCF obtained from deep periodontal pockets presented high levels of inflammatory cytokines. Clinical

periodontal parameters were analyzed by Mann-Whitney test and showed that T2DM and periodontitis groups exhibited higher levels of Probing depth. Within the limitation of small sample size, it was concluded that inflammatory mediators in GCF are dependent to the local response and do not correlate with the diabetic status.

Geng Y et al. (2018)[52] investigated the influences of IL-10 polymorphisms on the susceptibility to chronic periodontitis (CP) and aggressive periodontitis (AP), and their possible role in the quantity of subgingival bacteria Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis. Total 266 patients were recruited and are divided into 3 groups: CP patients (n=92), AP patients (n=83) and periodontal healthy controls (n=91). Serum IL-10 concentration was assessed by enzyme-linked immunosorbent assay (ELISA). Gene polymorphisms were determined by multiplex SNaPshot technique and bacterias were quantified by real-time polymerase chain reaction with TaqMan MGB probes. Taking into consideration demographic characteristics and periodontal status, IL-10-592 AA, -819 TT and ATA/ ATA genotype occurred more frequently in patients with CP than in healthy controls. In homozygous ATA/ATA carriers in CP cases, detected higher quantity of subgingival A. actinomycetemcomitans and lower serum IL-10 levels. These findings indicate that variants in IL-10 promoter gene were not only associated with predisposition to chronic periodontitis but also affected the subgingival number of A. Actinomycetemcomitans in a Chinese Han population. This study supports the hypothesis that complex interactions between the host genetic variants and the subgingival microbiota are at the basis of susceptibility to periodontitis.

Materials and Methods

The present split-mouth randomized clinical trial was carried out to evaluate the effect of PDT as an adjunct to NSPT on GCF IL-6, IL-8 and IL-10 levels in chronic periodontitis patients.

A total number of 21 patients (14 females and 7 males) affected with moderate to severe chronic periodontitis and comprising of both the sexes above 35 years of age were recruited for the study, which was conducted from September 2018 to October 2019 in the Department of Periodontology and Implantology, of our Institute. The study design was reviewed and approved by the Institutional Ethics Committee and was in accordance with the Helsinki Declaration. A special proforma was designed so as to have systematic and methodological recording of observation and information. This included a detailed case history, clinical examination, radiographic evaluation, periodontal indices and written consent of the patient.

Patient selection criteria

A dental and medical history was recorded for the selected patients. The intraoral examination was conducted by a single examiner which included Probing pocket depth (PPD), Clinical attachment level (CAL), Plaque index (PI) [Silness and Loe 1964][53], Gingival index (GI) [Loe and Silness, 1963][54], Bleeding on Probing (BOP) and radiographic evidence of bone loss.

Inclusion criteria

Systemically healthy patients, aged > 35 years, with at least 20 natural teeth in the oral cavity. Patients affected and diagnosed with moderate to severe chronic periodontitis, as assessed with Probing Depth (PD) ≥ 5mm and Clinical Attachment Loss (CAL) ≥ 5mm with alveolar bone loss affecting > 30% of the teeth as detected on radiograph at the time of initial diagnosis and the patients exhibiting similar periodontal pocket depths in contralateral premolars and molars were included in the study.

Diagnosis of Chronic periodontitis based on the classification by American Academy of Periodontology, 1999.[55]

Exclusion criteria

• Patients with medical disorders such as cardiovascular or renal disease, poorly controlled diabetes, liver diseases, malignancy, radiotherapy, or allergy to toluidine blue O.

• Patients with recent history or presence of acute or chronic infection.

• Individuals with history of tobacco chewing and smoking.

• Pregnant and lactating females.

• Patients with history of periodontal therapy or antibiotic treatment or any other anti-inflammatory medication within previous 6 months.

Study groups

A total number of 21 patients affected with moderate to severe chronic periodontitis and comprising of both the sexes above 35 years of age, visiting the Department of Periodontology and Implantology, were included in the present study. Sample size calculated was 20. However 23 patients were recruited for the study out of which 2 were dropped out during follow-up period.

The sample size was calculated using formula:

$$N = \frac{2\,(Z_\alpha + Z_\beta)^2\, S^2}{d^2}$$

Z_α – 1.96 for α=0.05

Z_β – 0.84 for β=0.2 i.e. 80% power

$$S^2 = \frac{S_1^2 + S_2^2}{2}$$

d= mean difference

Randomisation

All the patients fulfilling the inclusion criteria were recruited for the study, where in two sites per patient were randomly allocated to one of the two treatment modalities. Randomisation was done using computer generated random number table. Two contralateral sites (premolar and molar) per patient were randomly divided into:

Control sites (NSPT): Sites that were treated with NSPT only.

Test sites (NSPT + PDT): Sites that were treated with NSPT along with PDT.

Following periodontal parameters were recorded: Plaque Index (PI) [Silness and Loe 1964], Gingival index (GI) [Loe and Silness 1963], Bleeding on probing (BOP), Probing pocket depth (PPD), Clinical attachment level (CAL). Full mouth PI and GI were recorded while BOP, PPD and CAL were recorded from test and control sites at baseline, 1 & 3-months after therapy. All the patients were evaluated for the GCF levels of IL-6, IL-8 and IL-10 at baseline and 3 months. Each patient was explained about the treatment procedure to be performed and an informed consent was obtained prior to beginning of the study.

Armamentarium

Following material and armamentarium was used for the assessment of clinical parameters, for the collection of GCF and for clinical procedure. **(Color plates I, II, III)**

For examination of the patient

1. Mouth mirror
2. Explorer
3. UNC -15 (Hu-Freidy) periodontal probe.
4. Tweezer
5. Kidney tray
6. Disposable gloves
7. Disposable face mask
8. Acrylic stents

For collection of GCF sample

1. 5µl micro capillary pipette
2. Eppendorf tube
3. Sterilized cotton rolls.

For clinical procedure

1. Ultrasonic scaling device & tips
2. Hand scaling instruments
3. Laser machine and components
4. Saline irrigation syringe
5. Dye applicator

Site selection and GCF collection

GCF samples were obtained from contra lateral premolars & molars of each patient after gently drying the area with a blast of air, supragingival plaque was removed using a hand scaler without touching the marginal gingiva. The area was isolated using sterile gauze to prevent contamination by saliva and GCF was collected by placing a micro capillary pipette at the entrance of the gingival sulcus, gently touching the marginal gingiva. Around 2- 3 µl volume was collected using calibration on 1-5µl calibrated volumetric micropipettes. GCF samples were collected from sites with PPD > 5mm, at baseline and 3 months.

Each sample collection was allotted a maximum of 10 minutes and sites that did not express any GCF within the allotted time were excluded. This was to ensure atraumatism and micropipettes that were suspected to be contaminated with blood and saliva were excluded from the study. Collected GCF samples were immediately transferred to airtight plastic vials (eppendorf tubes) and stored at -20°C until assayed.

Assessment of periodontal and clinical parameters

1. Plaque Index (PI): (Silness and Loe, 1964)[53]

PI was examined in the scoring units of teeth: distofacial, facial, mesiofacial and palatal/lingual surfaces surfaces of the selected index teeth. The teeth selected as index teeth were: 16- Maxillary Right First Molar

12- Maxillary Right Lateral Incisor

24- Maxillary Left First Premolar

36- Mandibular Left First Molar

32- Mandibular Left Lateral Incisor

44- Mandibular Right First Premolar

A mouth mirror and dental explorer were used to assess Plaque index.

The criteria for scoring were as follows

SCORE	CRITERIA
0	No plaque in gingival area
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque was recognized only by running a probe across the tooth surface
2	Moderate accumulation of soft deposits within the gingival pocket and on the gingival margin and/or adjacent tooth surface, which could be seen by the naked eye.
3	Abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface

A plaque index per person was obtained by adding all of the plaque scores and dividing by the number of surfaces examined.

$$PI = \frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$$

Plaque scores	Rating
0	Excellent
0.1-0.9	Good
1.0- 1.9	Fair
2.0- 3.0	Poor

2. Gingival Index (GI): (Loe and Silness, 1963)[54]

This index is for assessing the severity of gingivitis in four possible areas. The severity of gingivitis was scored on mesial, distal, buccal and palatal/lingual surfaces of the selected index teeth. The teeth selected as index teeth were:

16- Maxillary Right First Molar

12- Maxillary Right Lateral Incisor

24- Maxillary Left First Premolar

36- Mandibular Left First Molar

32- Mandibular Left Lateral Incisor

44- Mandibular Right First Premolar

A UNC-15 periodontal probe was used to assess the bleeding potential of the gingival margin according to the following criteria-

SCORE	CRITERIA
0	Normal gingiva
1	Mild inflammation, slight change in color, slight edema, no bleeding on palpation
2	Moderate inflammation, redness, edema and glazing, bleeding on palpation
3	Severe inflammation, marked redness and edema, ulcerations, tendency of spontaneous bleeding

The scores of all the surfaces were added and divided by number of surfaces examined which provided the gingival index score per person.

GI = Total scores of all teeth / Total number of teeth examined

Gingival scores	Condition
0.1 to 1.0	Mild gingivitis
1.1 to 2.0	Moderate gingivitis
2.1 to 3.0	Severe gingivitis

3. Bleeding on Probing (BOP): BOP was assessed by presence or absence of bleeding, up to 30 seconds after probing.[56]

4. Probing Pocket Depth (PPD):

It was measured using Hu Friedy UNC-15 periodontal probe on 4 sites (mid-buccal, mid-palatal/mid-lingual, mesial, distal) of all present teeth. PPD was measured as the distance from the crest of marginal gingival to the depth of the periodontal pocket or gingival sulcus. Patients were diagnosed with chronic periodontitis if they exhibited PPD ≥ 5mm and CAL ≥ 5mm at multiple sites.

5. **Clinical Attachment Level (CAL):**

It was measured using Hu Friedy UNC-15 periodontal probe on 4 sites (distal, buccal, mesial, lingual/palatal) from the cementoenamel junction (CEJ) to the base of the periodontal pocket of all the present teeth. This was calculated by measuring the distance from CEJ to the gingival margin and subtracting this value from probing depth measurement. Patients were diagnosed with chronic periodontitis if they exhibited CAL ≥ 5mm at multiple sites.

Preparation of acrylic stents

The custom made occlusal acrylic stents were used to standardize the probe angulation and position. Occlusal stents were fabricated with cold cured acrylic resin on a cast

model obtained from an alginate impression. The occlusal stents covered the occlusal surface of the tooth being treated and occlusal surfaces of at least one tooth in mesial and distal directions. Stents also extended apically on the buccal and lingual surfaces of the teeth. A groove (guide plane) was made on the stent in relation to each involved tooth to guide the periodontal probe while taking measurements. This technique provided a fixed reference point and fixed angulations for measurements at each site.

Study settings

After clinical examination and sample collection, one site was randomly assigned to one of the treatment modalities:

a. Non-Surgical Periodontal Therapy (NSPT) alone

b. Non-Surgical Periodontal Therapy (NSPT) with adjunctive photodynamic therapy (NSPT+ PDT)

The NSPT comprised of scaling and root planing along with oral hygiene instructions. After allocation of sites, full-mouth scaling and root planing was performed for all patients. Mechanical instrumentation was performed using Gracey curettes and ultrasonic scaler tips.

Clinical procedure

21 patients affected with moderate to severe chronic periodontitis and comprising of both the sexes above 35 years of age were included in the present study. All the selected patients were received thorough scaling and root planing. Then selected sites in each patient were randomly assigned to Control site (NSPT alone) and Test site (NSPT+ PDT). Each patient was explained about the treatment procedure to be performed and an informed consent was obtained prior to beginning of the study.

The PDT system consisted of three basic components:

1. A laser base station incorporating a low power (<2 W), continuous-wave diode laser (Biolitec, Germany) operating at 810-nm over a 60-second pre-programmed treatment cycle with the total energy as 6 J.
2. An autoclaveable handpiece connected to the laser via fiberoptic cable.
3. A treatment kit composed of a single-use light diffusing tip, a blunt-ended irrigation needle and a pre-filled syringe containing photosensitizer solution (0.01% toluidine blue O (TBO) USP in a buffered, isotonic, viscosity-modified base).[44]

Toluidine blue O (2-methyl-3-dimethylamino-7-amino-phenothiazin-5-iumchloride) is a phenothiazinium group planar cationic dye that has anti-tumor activity. TBO is widely known to have antimicrobial properties when used as photosensitizers. TBO is an effective photosensitizer against planktonic bacterial and fungal growth, which has shown to reduce the cell viability of many microorganisms.[57]

For the sites receiving PDT, the periodontal pocket was filled with the TBO photosensitizer using a syringe in a coronal direction starting in the most apical portion. A photosensitization period of 60 seconds was used. After that, the pocket was irrigated with distilled water and irrigated pocket was then illuminated via the laser handpiece where it was moved in coronal direction using sweeping motion for 1 minute followed by additional vertical movements in apical and coronal directions for 30 seconds.[31] **(Color plate IV)**

All the participants were given oral hygiene instructions. No systemic antibiotics or anti-inflammatory medications were administered.

Recall visits (Color plates V, VI)

The patients were re-evaluated at 1 and 3 months post therapy. Using acrylic stents, the clinical parameters were recorded at both recall visits with UNC-15 periodontal probe. Collection of GCF samples were done at 3-months visit. Patients with PPD > 5 mm and CAL > 5 mm at 3-months visit were undertaken for surgical procedure.

Biochemical assay

Laboratory armamentarium for assessment of biochemical parameters (Color plate VII)

1. Calibrated, volumetric transfer pipettes with 0-5 µl range, 5-50 µl range, 50-200 µl range and 200-1000 µl range
2. Eppendorf tubes
3. Distilled water
4. Beakers, measuring cylinder
5. Sterile gloves
6. Sterile cotton
7. ELISA kits (Krishgen Biosystems Human IL-6, IL-8, IL-10)

Laboratory equipment (Color plates VIII, IX)

1. -80°C deep freezer (REMI Equipments Pvt. Ltd.)
2. Vortex mixer (CM 101, REMI Equipments Pvt. Ltd.)
3. Automated Microplate Washer (LISA wash microplate washer)
4. ELISA reader (LISA Microplate reader, REMI Equipments Pvt. Ltd.)

Evaluation of IL-6 from GCF

Samples were assayed for Human Interleukin-6 (IL-6) levels using commercially available ELISA (Enzyme-linked immunesorbent assay) Krishgen Human IL-6 ELISA Kit. Samples were assessed according to the instruction manual at the Biochemistry Department.

Materials required

1. Microtiter Coated Plate (12X8 wells) – 1 no.

2. Recombinant Human IL-6 Standard – 1 vial

3. Human IL-6 Biotin Conjugated Detection Antibody – 1 vial

4. Concentrated Avidin Horseradish Peroxidase - 1 vial

5. Wash Buffer (20X) – 25ml

6. Assay Diluent (5X) – 10ml

7. TMB Substrate – 12ml

8. Stop Solution – 12ml

Additional materials required

1. Microplate Reader able to measure absorbance at 450nm.

2. Adjustable pipettes to measure volumes ranging from 50µl to 1000µl.

3. Deionized (DI) water.

4. Wash bottle or automated microplate washer.

5. Semi-Log graph paper or software for data analysis.

6. Tubes to prepare standard/sample dilutions.

7. Timer.

8. Absorbent paper.

All the reagents were diluted immediately prior to use as instructed by the manufacturer.

Assay procedure

1. All reagents and samples were brought to room temperature (18 - 25ºC) before use.
2. Around 100 µl of each standard and sample were added to appropriate wells, covered and incubated for 2 hours at room temperature.
3. The solution was aspirated and washed 4 times with Wash Buffer (1X) and residual buffer was blotted by firmly tapping plate upside down on absorbent paper.
4. 100µl of diluted Detection Antibody solution was added to each well. The plate was sealed and incubated for 1 hour room temperature (18-25°C).
5. Plate was washed 4 times with Wash Buffer (1X) as in step 3.
6. 100µl of diluted Avidin-HRP solution was added to each well, then again the plate was sealed and incubated for 30 minutes room temperature (18-25°C).
7. Plate was washed 4 times with Wash Buffer (1X) as in step 3. For this final wash, the wells were soaked in Wash Buffer for 30 seconds to 1 minute for each wash.
8. Then 100µl of TMB Substrate solution was added and the plate was incubated in the dark for 20-minutes. Positive wells turned bluish in color.
9. Reaction was stopped by adding 100µl of Stop Solution to each well. Positive wells turned from blue to yellow.
10. The absorbance was read at 450 nm within 30 minutes of stopping reaction.

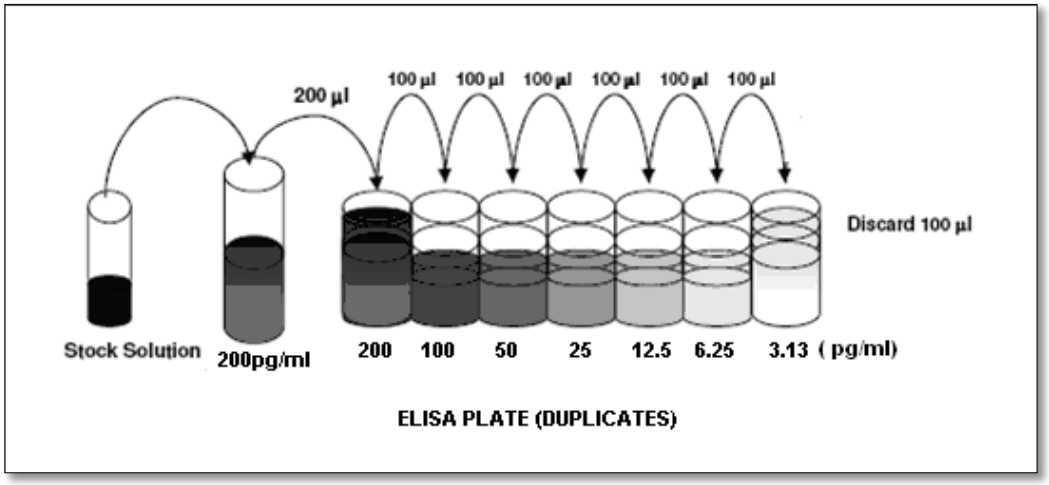

Fig. 1: Preparation of standard for IL-6 assay

Calculation of Results

The mean absorbance was determined for each set of duplicate or triplicate standards and samples. The mean absorbance of the zero standards (background) was subtracted from each well. The standard curve was plotted on Semi-Log graph paper, with concentration of the standards on the X-axis and the optical densities of each standard on the Y-axis. The best fit straight line was drawn through the standard points. To determine the unknown cytokine concentrations the unknown mean absorbance value on the y-axis were found and a horizontal line was drawn to the standard curve. At the point of intersection, a vertical line was drawn to the x-axis and the cytokine concentration was read.

Evaluation of IL-8 from GCF

Samples were assayed for Human Interleukin-8 (IL-8) levels using commercially available ELISA (Enzyme linked immune-sorbent assay) Krishgen Human IL-8 ELISA Kit. Samples were assessed according to the instruction manual at the Biochemistry Department.

Materials Provided

1. Microtiter Coated Plate (12X8 wells) – 1 no.

2. Recombinant Human IL-8 Standard – 2 vials

3. Human IL-8 Biotin Conjugated Detection Antibody – 1 vial

4. Concentrated Streptavidin Horseradish Peroxidase - 1 vial

5. Wash Buffer (20X) – 25ml

6. Assay Diluent – 50ml

7. TMB Substrate – 12ml

8. Stop Solution – 12ml

Additional materials required

1. Microplate Reader able to measure absorbance at 450nm.

2. Adjustable pipettes to measure volumes ranging from 50µl to 1000µl.

3. Deionized (DI) water.

4. Wash bottle or automated microplate washer.

5. Semi-log graph paper or software for data analysis.

6. Tubes to prepare standard/sample dilutions.

7. Timer.

8. Absorbent paper.

All the reagents were diluted immediately prior to use as instructed by the manufacturer.

Assay procedure

1. All the reagents were brought to room temperature prior to use. All standards and samples were run in duplicate or triplicate.
2. Around 100 µl of each standard and sample were added to appropriate wells, covered and incubated for 2 hours at room temperature.
3. The solution was aspirated and washed 4 times with Wash Buffer (1X) and residual buffer was blotted by firmly tapping plate upside down on absorbent paper.
4. 100µl of diluted Streptavidin-HRP solution was added to each well. The plate was sealed and incubated for 30 min at 37°C.
5. Plate was washed 4 times with Wash Buffer (1X) as in step 3.
6. 100µl of TMB Substrate solution was added to each well, then incubated in the dark for 30 minutes at 37°C. Positive wells turned bluish in color.
7. Reaction was stopped by adding 100µl of Stop Solution to each well. Positive wells turned from blue to yellow.
8. The absorbance was read at 450 nm within 30 minutes of stopping reaction.

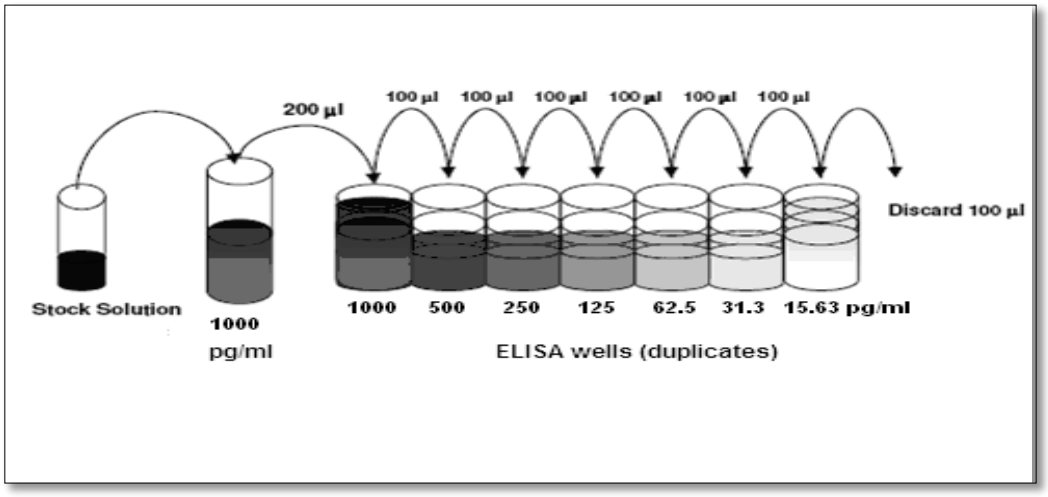

Fig. 2: Preparation of standard for IL-8 assay

Calculation of Results

The mean absorbance was determined for each set of duplicate or triplicate standards and samples. The mean absorbance of the zero standards (background) was subtracted from each well. The standard curve was plotted on Semi-Log graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. The best fit straight line was drawn through the standard points. To determine the unknown cytokine concentrations the unknowns mean absorbance value on the y-axis were found and a horizontal line was drawn to the standard curve. At the point of intersection, a vertical line was drawn to the x-axis and the cytokine concentration was read.

Evaluation of IL-10 from GCF

Samples were assayed for Human Interleukin-10 (IL-10) levels using commercially available ELISA (Enzyme linked immune-sorbent assay) Krishgen Human IL-10 ELISA Kit. Samples were assessed according to the instruction manual at the Biochemistry Department.

Materials Provided

1. Microtiter Coated Plate (12 X 8 wells) – 1 no.

2. Recombinant Human IL-10 Standard – 2 vials

3. Human IL-10 Biotin Conjugated Detection Antibody – 1 vial

4. Concentrated Streptavidin Horseradish Peroxidase – 1 vial

5. Wash Buffer (20X) – 25ml

6. Assay Diluent – 50ml

7. TMB Substrate – 12ml

8. Stop Solution – 12ml

Additional materials required

1. Microplate Reader able to measure absorbance at 450nm.

2. Adjustable pipettes to measure volumes ranging from 50µl to 1000µl.

3. Deionized (DI) water.

4. Wash bottle or automated microplate washer.

5. Semi log graph paper or software for data analysis.

6. Tubes to prepare standard/sample dilutions.

7. Timer.

8. Absorbent paper

All the reagents were diluted immediately prior to use as instructed by the manufacturer.

Assay procedure

1. All the reagents were brought to room temperature prior to use. All standards and samples were run in duplicate or triplicate.
2. Around 100 µl of each standard and sample were added to appropriate wells, covered and incubated for 2 hours at room temperature.
3. The solution was aspirated and washed 4 times with Wash Buffer (1X) and residual buffer was blotted by firmly tapping plate upside down on absorbent paper.

4. 100µl of diluted Streptavidin-HRP solution was added to each well. The plate was sealed and incubated for 30 min at 37°C.
5. Plate was washed 4 times with Wash Buffer (1X) as in step 3.
6. 100µl of TMB Substrate solution was added to each well, then incubated in the dark for 15-30 minutes at 37°C and the wells were observed at every 5mins time interval. Positive wells turned bluish in color.
7. Reaction was stopped by adding 100µl of Stop Solution to each well. Positive wells turned from blue to yellow.
8. The absorbance was read at 450 nm within 30 minutes of stopping reaction.

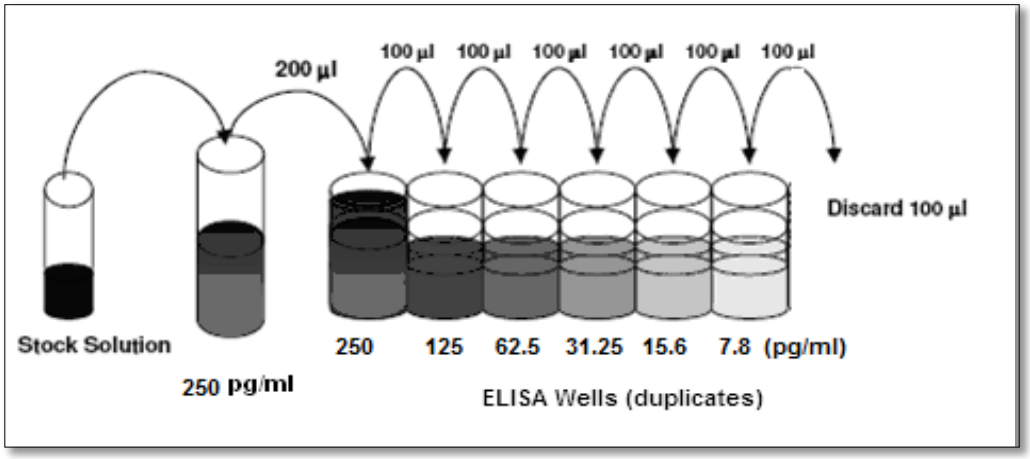

Fig 3: Preparation of standard for IL-10 assay

Calculation of Results

The mean absorbance was determined for each set of duplicate or triplicate standards and samples. The mean absorbance of the zero standards (background) was subtracted from each well. The standard curve was plotted on Semi-Log graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. The best fit straight line was drawn through the standard points. To determine the unknown cytokine concentrations the unknowns mean absorbance value on the y-axis were found and a

horizontal line was drawn to the standard curve. At the point of intersection, a vertical line was drawn to the x-axis and the cytokine concentration was read.

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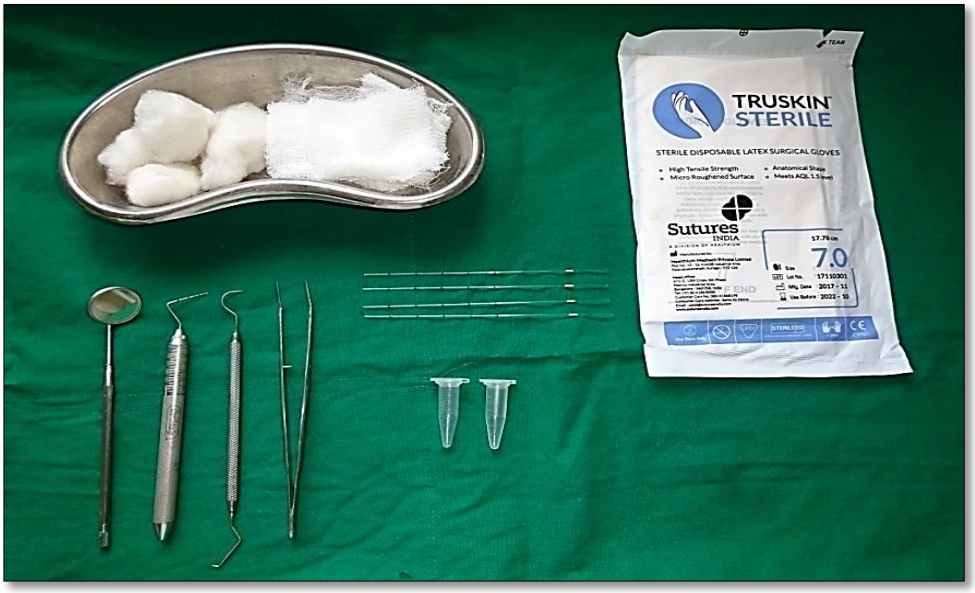
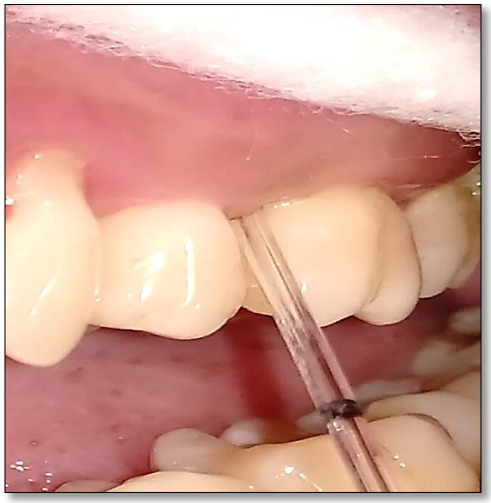
Color Plate I

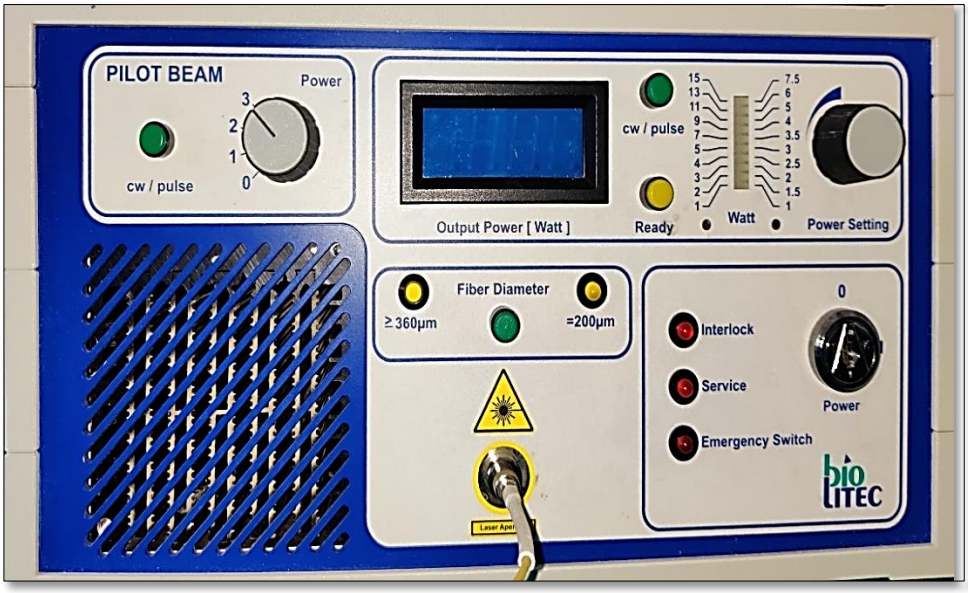


Armamentarium for clinical examination and GCF collection



GCF Collection

Color Plate II



Laser machine

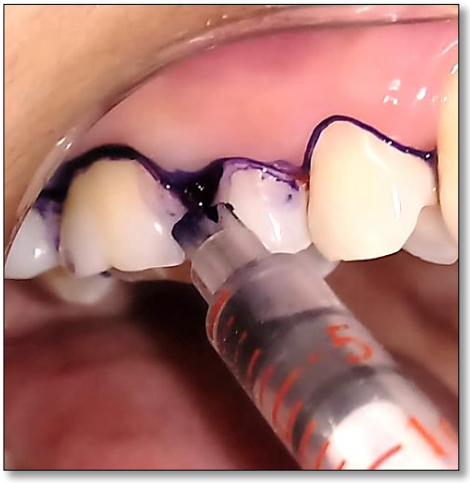
Components

Color Plate III

Dye applicator

Color Plate IV

Clinical procedure

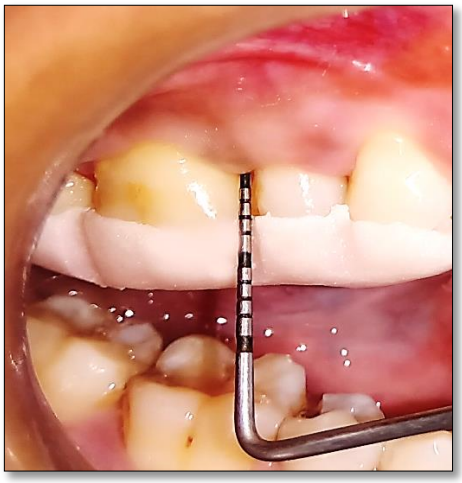


Dye application

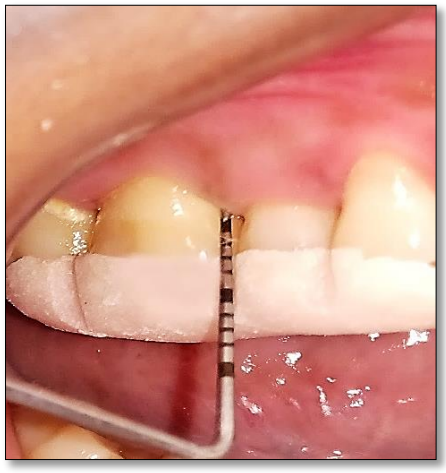
Laser application

Color Plate V

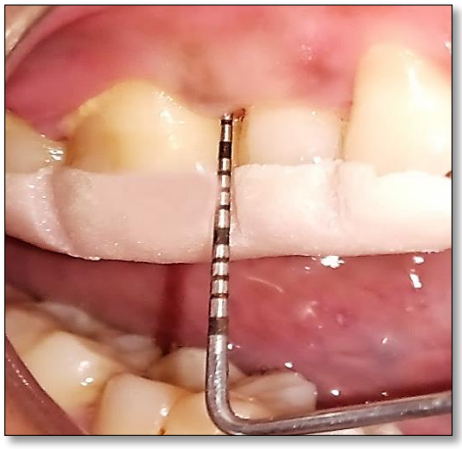
Test site



Probing depth at baseline



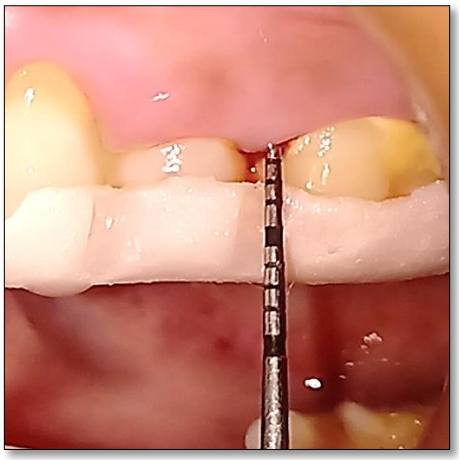
Probing depth at 1 month



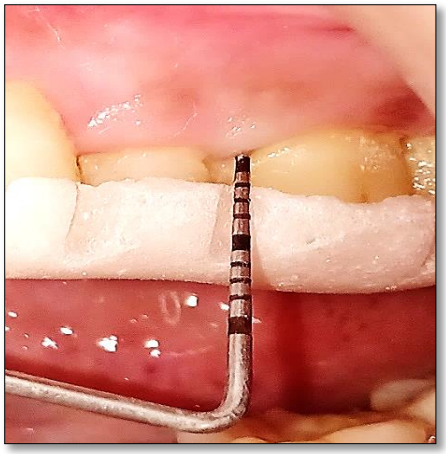
Probing depth at 3 months

Color Plate VI

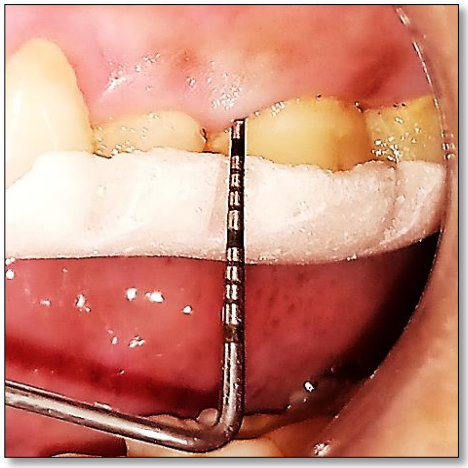
Control site



Probing depth at baseline



Probing depth at 1 month



Probing depth at 3 months

Color Plate VII

ELISA kits for IL-6, IL-8 and IL-10

Color Plate VIII

Deep freezer

Vortex Mixer

Color Plate IX

Micro-plate Washer

Micro-plate Reader

Results

The present randomized controlled trial was aimed to evaluate the efficacy of PDT as an adjunct to NSPT on the levels of GCF IL-6, IL-8 & IL-10 levels in chronic periodontitis. Owing to multifactorial nature of periodontitis and limitations of conventional treatment modalities, led to the introduction of photodynamic therapy. Various biomarkers have been evaluated in chronic periodontitis till date, amongst which IL-6, IL-8 are pro-inflammatory & IL-10 is an anti-inflammatory cytokine. We hypothesized their possible link with chronic periodontitis and variation in their levels after treatment with PDT as an adjunct to NSPT and NSPT alone.

For this research we had recruited patients which were examined clinically and biochemically and then categorized into two groups. IL-6, IL-8 & IL-10 levels were estimated using ELISA in GCF to evaluate and compare their levels at baseline and 3 months after PDT and NSPT.

Incorporation of clinical and biochemical techniques enabled us to fulfil our above objectives.

Statistical analysis

The data on demographic parameters were summarized according to scale of measurement. The clinical parameters like plaque and gingival index were expressed in terms of mean and standard deviations and compared across times using repeated measure analysis of variance. Other parameters like BOP, PPD and CAL were

compared between test and control sites using paired t-test. Also, the comparisons were performed for each parameter across time at each site using repeated measure analysis of variance. The cytokines viz., IL-6, IL-8 and IL-10 were also compared between two sites using paired t-test. The comparison of each parameter at each site was also performed between two time points using paired t-test. Further, Pearson's correlation coefficient was also obtained to determine the relationship of parameters (PPD, CAL and cytokines) between different times. The analysis was performed independently for molar and premolar specimens. All the analyses were performed using SPSS ver 20.0 (IBM Corp, ARMONK USA) and the statistical significance was tested at 5% level.

The descriptive statistics for the demographic characteristics of patients are shown in **Table 1**. The mean age of patients was 44.95 ± 8.51 years. Out of 21, maximum i.e. 14 (66.66%) were females and 7 (33.34 %) were males. All the patients returned for follow-up evaluation at 1 and 3 months post-treatment. During the course of study, no allergic reaction was reported, indicating the biocompatibility of toluidine blue O dye.

Table 2 provides a comparison of Plaque index and Gingival index across time. Baseline full mouth plaque score was 2.39±0.51, while at 1 month it decreased to 1.53±0.53 and at 3 months the mean PI was 0.97±0.42. The plaque index showed statistically significant difference across time as indicated by a p-value < 0.0001 using repeated measure ANOVA. The baseline plaque index was significantly higher than 1 month and 3 months**. (Graph 1)**

The mean gingival index dropped from 2.09±0.59 at baseline to 1.20±0.38 at 1 month and to 0.98±0.43 at 3 months. The GI showed statistically significant difference across

time with a p-value < 0.0001 by using repeated measures of ANOVA. The baseline index was significantly higher than that of 1 month and 3 months. **(Table 2) (Graph 1)**

Table 3 provides the comparison for BOP between two sites as well as across time at each site. At baseline, in test site mean BOP was 64.10±7.67% while in control site it was 64.43±7.58% and there was no significant difference between two sites (p=0.888). At 1 month, mean BOP decreased to 41.10±3.58% for test site and to 45.29±3.90% for control site. BOP showed statistically significant reduction in test and control sites at 1month (p=0.001) using paired t-test. At 3 months, mean BOP was 38.00±3.62% for test site and 46.81±4.33% for control site. BOP showed a statistically significant mean difference between two sites with a p-value of < 0.0001 at 3rd month, using paired t-test. At the control site, the BOP was higher as compared to the test site. The comparison of BOP across time in both the sites showed statistically significant differences as indicated p-value < 0.0001. The baseline BOP measurements were significantly higher than that of 1st and 3rd months. **(Graph 2)**

Table 4 provides the comparison for PPD between two sites as well as across time at each site for premolar and molar. For Premolar, in test site the mean PPD at baseline was 5.23±0.65 mm while in control site it was 5.14±0.70 mm. At baseline there was no significant difference in PPD between test and control site (p=0.444). At 1month, the mean PPD reduction was 4.10±0.64 mm for test site and 4.37±0.69 mm for control site. PPD showed statistically significant mean difference between two sites at 1month, with a p-value of 0.021, using paired t-test. At 3 months, the mean PPD reduction was 3.42±0.63 mm for test site and 3.66±0.66 mm for control site. Using paired t-test, PPD showed statistically significant mean difference between two sites at 3 months (p-value

of 0.022). The comparison of PPD across three different time points i.e at baseline, 1 and 3 months suggested statistically significant differences with a p-value < 0.0001, at both the sites, using repeated measure ANOVA**. (Graph 3)**

For molar, in test site, the mean PPD at baseline was 5.92±0.71 mm which reduced significantly to 4.82±0.71 mm at 1 month and to 4.12±0.71 mm at 3 months (p-value < 0.0001). In control site, the mean PPD at baseline was 5.78±0.54 mm which reduced significantly to 4.80±0.67 mm at 1 month and to 4.15±0.68 mm at 3 months (p-value < 0.0001). The comparison of mean PPD across three different time points suggested statistically significant differences with a p-value < 0.0001, at both the sites, using repeated measure ANOVA. At baseline, 1 and 3 months, PPD showed statistically insignificant mean difference between test and control sites, though reduction was more in test site at 1 and 3 months. **(Table 4)(Graph 4)**

Correlation of PPD between baseline and 3 months for molar and premolar teeth at test and control sites is shown in scatter plot **(Graph 10)**. The relationship of PPD between baseline and 3 months was studied using Pearson's correlation coefficient for premolar and molar independently. For premolar, at test site, the coefficient was 0.993 suggesting a strong positive correlation between the observations at two time points, which was statistically significant with a p-value < 0.0001. At control site, the coefficient was 0.992 suggesting a strong positive correlation between the observations at two time points, which was statistically significant with a p-value < 0.0001. For molars, the correlation was also strongly positive with coefficients 0.998 and 0.981 at test and control sites respectively, and significant with a p-value < 0.0001. **(Graph 10)**

Table 5 provides the comparison for CAL between two sites and across time at each site for premolar and molar. For Premolar, in test site, the mean CAL at baseline was

6.67±0.91 mm, followed by 5.55±0.92 mm at 1 month and 4.72±0.87 mm at 3 months (p-value < 0.0001). In control site, the mean CAL at baseline was 6.46±0.93 mm, 5.67±0.93 mm at 1 month and 5.06±0.93 mm at 3 months (p-value < 0.0001). The comparison of CAL at baseline, 1 and 3 months suggested statistically significant differences with a p-value < 0.0001, at both the sites, using repeated measure ANOVA. However, at baseline, 1 and 3 months, CAL showed statistically insignificant mean difference between test site and control site, whereas CAL gain was more in test site. **(Graph 5)**

For molar, in test site, the mean CAL at baseline was 7.58±1.09 mm, followed by 6.48±1.09 mm at 1 month and 5.78±1.09 mm at 3 months (p-value < 0.0001). In control site, the mean CAL at baseline was 7.52±1.47 mm, 6.80±1.51 mm at 1 month and 6±1.50 mm at 3 months (p-value < 0.0001). The comparison of CAL at baseline, 1 and 3 months suggested statistically significant differences with a p-value < 0.0001, at both the sites, using repeated measure ANOVA. Though gain in CAL was more in test site, CAL showed statistically insignificant mean difference between test site and control site at 3 different time points. **(Table 5) (Graph 6)**

Correlation of CAL between baseline and 3 months for molar and premolar teeth at test and control sites is shown in scatter plot **(Graph 11)**. The relationship of CAL between baseline and 3 months was studied using Pearson's correlation coefficient for premolar and molar independently. For premolar, at test site, the coefficient was 0.992 suggesting a strong positive correlation between the observations at two time points, which was statistically significant with a p-value < 0.0001. At control site, the coefficient was 0.997 suggesting a strong positive correlation between the observations at two time points, which was statistically significant with a p-value < 0.0001. For molars, the

correlation was also strongly positive with coefficients 0.998 and 0.997 at test and control sites respectively, and significant with a p-value < 0.0001. **(Graph 11)**

Table 6 provides the comparison for IL-6, IL-8 and IL-10 between two sites and also between two time points at each site. At baseline, mean IL-6 level at test site was 8.64±0.64 pg/ml and at control site was 8.48±0.70 pg/ml while at 3 months, mean IL-6 level decreased significantly in test site (4.29±0.67 pg/ml) and in control site (5.83±0.65 pg/ml). IL-6 showed a statistically insignificant mean difference between two sites at baseline, while a significant difference was observed at 3 months with a p-value < 0.0001 using paired t-test. For the test site and control site, the parameter showed statistically significant mean difference at 3 months as indicated by p-value < 0.0001 using paired t-test**. (Graph 7)**

At baseline, mean IL-8 level at test site was 456.53±9.42 pg/ml and at control site was 454.25±8.51 pg/ml while at 3 months, mean IL-8 level decreased significantly in test site (308.16±36.04 pg/ml) and in control site (379.51±7.32 pg/ml). IL-8 showed a statistically insignificant mean difference between two sites at baseline, while a significant difference was observed at 3 months with a p-value < 0.0001 using paired t-test. For the test site and control site, the parameter showed statistically significant mean difference at 3 months as indicated by p-value < 0.0001 using paired t-test. **(Table 6) (Graph 8)**

At baseline, mean IL-10 level at test site was 11.77±0.75 pg/ml and at control site was 11.56±0.73 pg/ml, while at 3 months, mean IL-10 level increased significantly in test site (14.25±0.83 pg/ml) and in control site (12.48±0.82 pg/ml). IL-10 showed a statistically insignificant mean difference between two sites at baseline, while a significant difference was observed between test and control sites at 3 months with a p-

value < 0.0001, using paired t-test. For the test site and control site, the parameter showed statistically significant mean difference at 3 months as indicated by p-value < 0.0001 using paired t-test. **(Table 6) (Graph 9)**

Correlation of biochemical parameters between baseline and 3 months at test and control sites is shown in scatter plot **(Graph 12-14)**. The relationship of each cytokine between baseline and 3 months was studied using Pearson's correlation coefficient for each site independently. For IL-6, at test site, the coefficient was 0.960 suggesting a strong positive correlation between the observations at two time points, which was statistically significant with a p-value < 0.0001. At control site, the correlation coefficient was 0.991 indicating strong positive relationship, which was statistically significant with a p-value < 0.0001. **(Graph 12)**

For IL-8, at test site, the coefficient was -0.416 suggesting a mild negative correlation between the observations at two time points, which was statistically insignificant. At control site, the correlation coefficient was 0.869 indicating strong positive relationship, which was statistically significant with a p-value < 0.0001. **(Graph 13)**

For IL-10, at test site, the coefficient was 0.630 suggesting a moderately positive correlation between the observations at two time points, which was statistically significant with a p-value of 0.002. At control site, the correlation coefficient was 0.965 indicating strong positive relationship, which was statistically significant with a p-value < 0.0001. **(Graph 14)**

Discussion

In periodontitis, the interaction between periodontal pathogens and the host inflammatory– immune system is responsible for the loss of periodontal attachment, destruction of connective tissues, and resorption of alveolar bone.[58] Periodontitis is a public health problem, as it is highly prevalent and causes disability as well as social inequality. 743 million people are affected by periodontitis worldwide and hence was estimated as the sixth most prevalent disease globally, in 2010. Though, polymicrobial dysbiosis is the initiating factor, the pathogenesis of periodontitis is determined by the development of a chronic inflammatory host immune response. The nature and extent of this response are central determinants of the susceptibility to and progression of periodontitis.[59] For the past 30 years, the immune response underlying the pathogenesis of the disease has been studied. The concept that the immune system reacts against plaque microorganisms was supported by histological studies.[60]

There is abundant evidence that major tissue destruction in the established periodontitis lesion results from recruitment of host cells via activation of monocytes/macrophages, fibroblast, lymphocytes, and other cell types. Substantial efforts have been made to study the cytokines released from host cells when exposed to components of periodontopathic bacteria.[60] Soluble protein ‘messenger’ molecules produced by a variety of cells are “Cytokines” that transmit signals to other cells. A vital role played by the cytokines in initiating and sustaining the inflammatory immune response is by

stimulating the production of secondary mediators. A cascade of events evoked by these mediators, amplifies the inflammatory response and induces the production of enzymes which are responsible for the degradation of connective tissue and osteoclastic bone resorption. In fact, an imbalance between the pro-inflammatory and anti-inflammatory cytokines derived from Th1, Th2, Th17 and Treg lymphocyte subpopulations is suggested as being responsible for periodontal breakdown through cellular and humoral hyper-immune responses.[58]

Inflammatory mediators are not only important to the pathogenesis of periodontal diseases but also used as diagnostic markers. Pivotal cytokine which is involved in the regulation of host response to infection and tissue injury, is IL-6. It plays a fundamental role in differentiation of B-cell and in T-cell proliferation. Synergism between IL-6 and IL-1β, induces bone resorption. Because of its effect in inflammation and bone resorption by stimulatory activity of the osteoclasts, IL-6 is considered to be a critical parameter in periodontal research.[61,62] **Tamura et al. (1993)** reported that Excess production of IL-6 and (s)IL-6R may contribute to osteoclastic bone resorption in several metabolic bone diseases, such as rheumatoid arthritis, postmenopausal osteoporosis and multiple myeloma. IL-8 is an important chemokine of interest in periodontal diseases which is a potent chemo-attractant cytokine and activator of neutrophils in inflammatory regions. The unique coordinated expression of IL-8 enables the transit of neutrophils from the highly vascularized gingival tissue to the gingival crevice.[63]

A pleiotropic cytokine known for its immunosuppressive properties is IL-10. It has been implicated in the suppression of tissue destruction. The synthesis of proinflammatory

cytokines (IL-1, IL-6, IL-8 and TNF) is inhibited by IL-10, also enhances the production of the IL-1 receptor antagonist (IL-1ra) in polymorphonuclear leukocytes stimulated with lipopolysaccharide (LPS). Thus, it was suggested that IL-10 might have an important regulatory role in limiting the duration as well as extent of the acute inflammatory response. IL-10 increases the synthesis of tissue inhibitors of metalloproteinases in macrophages and suppresses the production of metalloproteinases. In addition, it also suppresses the differentiation of cells with resorptive function in hemopoietic cell cultures, representing an important matrix protective cytokine during inflammation.[58] **Stein et al. (1997)** suggested that gingival mononuclear cells extracted from adult periodontitis patients constitutively produced more IL-10 than gingival mononuclear cells derived from non-inflamed tissues.[64]

The clinical parameters like probing pocket depth, clinical attachment level, plaque index, gingival index, papillary bleeding index etc. are most common and universally used indicators for determining disease status. However, they provide information only about past periodontal tissue destruction and neither reveal current disease activity nor predict future activity due to low sensitivity and positive predictive value.[65] Therefore, several molecules have been tried as potential biomarker including cytokines, enzymes, receptors and other proteins. They have served to be an efficient, quick and objective diagnostic and monitoring method, with the ability to screen for susceptibility and diagnosis of periodontal disease, evaluate treatment response, predict future tissue destruction, and identify disease progression.[66] These biomarkers can be found in several biologic fluids such as GCF, blood, serum or plasma and saliva. Collection of GCF is non-invasive and therefore this approach has been extensively explored in the search for potential diagnostic biomarkers of periodontal disease. As a result of

interaction of GCF between the bacterial biofilm and the cells of the periodontal tissues, it appears as an attractive oral diagnostic fluid due to its ease of collection and allowing for sampling of multiple sites simultaneously.[67]

GCF is an inflammatory exudate that flows from the periodontal pocket (or gingival sulcus). Volumes are typically low in non-inflamed sites, and generally increases with increasing inflammation in the gingival and periodontal tissues. GCF is a complex mixture of substances derived from serum and from locally generated mediators of inflammation and tissue turnover/ breakdown. It also contains cellular components (neutrophils) and bacterial components. GCF plays a protective role in host–bacteria interactions, via a physical protective effect (dilution of bacteria and their products, plus outflow of fluid) as well as transportation of antibacterial substances into the pocket. The quantity of GCF flowing from a site is influenced not only by the degree of inflammation in the tissues but also by the extent of ulceration of the sulcular/ pocket epithelium.[68] Taking this into consideration this randomized controlled study was formulated to utilize GCF as an avenue for evaluating IL-6, IL-8 and IL-10 levels in periodontitis.

The ultimate goal of periodontal therapy is to eliminate supragingival and subgingival plaque as well as to arrest the progression of periodontal disease. Scaling and root planing (SRP) are considered as the "gold standard" for the treatment of periodontitis. Many studies have shown that following significant improvements after SRP, complete elimination of subgingival periodontal pathogens and irritants is not always possible. Similar challenges presented by residual pockets during SRP hence, additional therapeutic approaches to achieving periodontal health are required. Antibiotics are widely used to improve the results of mechanical debridement. Limitations of drug

resistance accompanied with the use of local and systemic medications have led to the popularity of antimicrobial photodynamic therapy (PDT) in the management of chronic periodontitis. PDT was introduced in 1904 as the light-induced inactivation of cells, microorganisms or molecules. This treatment modality is based on the principle that a photoactivatable substance (photosensitizer) is activated by the light of a particular wavelength. The transfer of energy causes the formation of free radicals of singlet oxygen, which exert destructive action on bacteria and their products.[69] The cytotoxic species generated by photodynamic therapy lead to inactivation of the membrane transport system and inhibition of plasma membrane enzyme activities.[70]

Various combinations of lasers and photosensitizing (PS) agents are used in antimicrobial photodynamic therapy. Toluidine blue O and methylene blue are most commonly used photosensitizing dyes, have similar chemical and physicochemical characteristics and have been used previously to detect mucosal tumors or atypical epithelia because they do not stain normal mucosa. They are the PS agents of choice for PDT because they have a pronounced cationic charge which helps them bind to the outer membrane of Gram-negative bacteria and penetrate bacterial cells, thereby indicating a high degree of selectivity for killing microorganisms compared with host mammalian cells. These dyes have been used in various concentrations with a residence time of 1 to 5 min in the periodontal pocket. After a resident period of 1 to 3 min, excess PS was flushed off so that it would not act as an optical shield during laser irradiation.[69]

In this method, diode lasers are commonly used. Wavelengths between 650-900 nm which are within the visible red light and near infrared, and have great influence on biological tissue are used. It is based on the fact that irradiation at a specific wavelength

is able to alter the cellular behavior, called as bio stimulation (or) bio modulation effect. This effect is achieved by acting on the on membrane calcium channels or cellular mitochondrial respiratory chain. An increase in cell metabolism and proliferation subsequently promoted by this action. In vivo and in vitro data suggest that PDT facilitates fibroblasts and keratinocyte cell motility, angiogenesis, collagen synthesis and growth factor release which lead to increased wound healing.[71]

Advantages of PDT in periodontal treatment.[72]

- As singlet oxygen and other free reactive oxygen species interact with several cell structures and different metabolic pathway and so chances of development of resistance to the PDT is less.
- As PDT is non-invasive local therapy, following application of a sensitizer, a light source which is delivered into the target area precisely via a fibre optic cable, so disturbances of the microflora at other sites and damage to the adjacent host tissues would not occur.
- PDT offers thorough irrigation and elimination of pathogens in inaccessible areas of periodontal pocket within short span of time, thus beneficial to both operator and the patient.
- After periodontal debridement the risk of bacteremia can be minimized.
- There is no need to prescribe antibiotics, therefore the possibility of side effects is avoided.
- There is no need to anaesthetize the area and destruction of bacteria is achieved in a very short period (<60 seconds).

Studies in the literature have suggested the effectiveness of photodynamic therapy in periodontitis; however, randomized controlled trials and systematic reviews have shown contrasting results regarding the efficacy of PDT.[69] To the best of our knowledge no study till date has evaluated GCF levels of IL-6, IL-8, IL-10 and clinical parameters in premolar and molar site along with photodynamic therapy.

Thus, the present study was aimed to evaluate the efficacy of Photodynamic therapy as an adjunct to non-surgical periodontal therapy on the levels of GCF IL-6, IL-8, and IL-10 levels in chronic periodontitis.

A total of 21 patients with chronic periodontitis above 35 years of age (mean age of 44.95 ± 8.51 years) of either sex who met the inclusion criteria were recruited and randomly assigned to each treatment group. A split-mouth design was employed because it allows the comparison of outcome between the treatments. It has the ability to greatly facilitate the interpretation of trials by minimizing the effects of inter-patient variability. Control sites were treated with NSPT alone while test sites were treated with PDT along with NSPT to evaluate their effect on GCF levels of IL-6, IL-8, and IL-10. Clinical parameters recorded were PI, GI, BOP, PPD and CAL at baseline and at 1 and 3 months post-treatment. GCF samples were collected and assessed using Krishgen Human IL-6, IL-8 and IL-10 ELISA kit for GCF at baseline and after 3 months.

During the course of study, there was no allergic reaction reported, indicating the biocompatibility of toluidine blue O dye. At baseline, no significant differences in any of the investigated parameters were observed between test sites and control sites indicating that the randomization process was effective.

There was a significant improvement in clinical parameters such as PI, GI, BOP, PPD and CAL after PDT and NSPT in all sites but test sites showed better improvement as compared to control sites. Levels of GCF IL-6 and IL-8 decreased and IL-10 increased after treatment but test sites showed more significant results.

Plaque control is essential for the long term stability of clinical outcomes. Bacterial plaque is a major and important factor in the etiology of periodontal destruction and successful therapy depends upon its removal subsequent to treatment. The decrease in PI was statistically significant at the end of 1 month and 3 months compared to baseline. The statistical decrease in plaque index scores in our study are in accordance with the finding of **Toker H et al. (2018)[22]**, **Christodoulides N et al. (2008)[14]**, **Theodoro LH et al. (2012)[35]**. **Toker H et al.[22]** evaluated the clinical measures after non-surgical periodontal treatment in chronic periodontitis patients and observed a significant decrease in full mouth plaque score after six weeks. Similarly, **Christodoulides N et al.[14]** and **Theodoro LH et al.[35]** on evaluating the efficacy of PDT in chronic periodontitis patients reported decrease in PI at the end of study period. The result of the above mentioned studies in accordance with our study accentuates the significance of repetitive reinforcement of oral hygiene instructions for maintenance of improved oral hygiene.

The GI reduction was found to be statistically significant, similar to the study by **Theodoro LH et al. (2012)[35]** who had evaluated the long-term clinical and microbiological effects of PDT associated with nonsurgical periodontal treatment and found reduction in both GI and PI scores. **Christodoulides N et al. (2008)[14]** evaluated the clinical and microbiologic effects of the adjunctive use of PDT to NSPT and

reported significantly greater improvement in full mouth bleeding score at 3 and 6 months.

At test site, mean BOP at baseline was 64.10±7.67% while it decreased to 41.10±3.58% at 1 month and 38.00±3.62% at 3 months. At control site, mean BOP at baseline was 64.43±7.58% which was dropped to 45.29±3.90% at 1 month and to 46.81±4.33% at 3 months. BOP showed a statistically significant mean difference between two sites across time with a p-value of < 0.0001. The BOP was higher at control site as compared to the test site. The possible explanation for higher reduction of BOP scores at PDT treated sites are the additional benefits provided by low level laser therapy to the surrounding tissues and cells during the tissue healing, favouring the repair of tissues and diminishing periodontal inflammation as a result of the potential biomodulatory effects, such as stimulation and proliferation of cells and the collagen synthesis. The statistical decrease in BOP score in our study are in accordance with the findings of **Campos GN et al. (2013)[34]** who evaluated the effect of a single photodynamic therapy (PDT) as an adjunct to scaling and root planing (SRP) in residual pockets in single-rooted teeth and reported significant reduction in BOP at test group 3 months post therapy. Similar reduction in the mean BOP in both PDT and Control group, with significantly greater reduction in PDT group at the end of 12 weeks were reported by **Sigusch BW et al. (2010)[73]**. Similar reduction in BOP has been reported by **Alwaeli HA et al. (2015)[12]** in previously untreated chronic periodontitis at 3 months, 6 months and 1 year.

However, a study done by **Theodoro LH et al. (2012)[35]** who evaluated the long-term clinical and microbiological effects of PDT associated with nonsurgical periodontal treatment and reported no significant difference in BOP score after a single session of

PDT and NSPT. The authors clarified that SRP effectively mechanically removed plaque and calculus, a single PDT session after SRP treatment did not result in a significant reduction in clinical signs of inflammation in patients with chronic periodontitis compared with SRP alone. The authors have attributed these differing results to the application of single session of PDT. While in our study, a statistically significant BOP reduction at test site could be due to application of two sessions of PDT along with NSPT.

For premolar, in test site mean PPD at baseline was 5.23±0.65 mm while it decreased to 4.10±0.64 mm at 1 month and 3.42±0.63 mm at 3 months. However in control site, mean PPD at baseline was 5.14±0.70 mm while it decreased to 4.37±0.69 mm at 1 month and 3.66±0.66 mm at 3 months. PPD showed statistically significant mean difference between two sites at 1 month and 3 months (p-value of 0.021 and 0.022 respectively) and also across three different time points with a p-value < 0.0001. These results were similar to those reported by **Alwaeli HA et al. (2015)[12]** who compared the adjunctive effect of PDT along with SRP and SRP alone and effects that can last for 1 year. Out of the total teeth included in the study, 92 % were single-rooted and 8 % were multi-rooted. Authors concluded that single application of PDT as an adjunctive therapy to SRP succeeded in PPD reduction at different time points (3, 6, and 12 months) compared with conventional SRP periodontal treatment alone. Similar reduction in the mean probing depths in both test and Control group with significantly greater reduction in test group at end of 3 months were reported by **Campos GN et al. (2013)[34]** who treated residual pockets in single-rooted teeth. It is known from the literature that several subject-related and tooth site-related factors may compromise the healing response to periodontal therapy. Periodontal pockets associated with furcation

involvements or in multi-rooted teeth responded less favourably to SRP than pockets at other tooth sites, such as non-molar teeth. Therefore, the morphology of the tooth as well as the pocket probing depth seem to influence the effectiveness of scaling and root planing.[74,75]

For molar, mean PPD at baseline for test and control sites was (5.92±0.71 mm, 5.78±0.54 mm) while it decreased to (4.82±0.71 mm, 4.80±0.67 mm) at 1 month and dropped to (4.12±0.71 mm, 4.15±0.68 mm) at 3 months. Using paired t-test, PPD showed statistically insignificant mean difference between test and control sites, at baseline (p-value=0.134), 1st month (p-value= 0.836) and 3rd months (p-value=0.722), whereas PPD reduction was more in test site. However, the comparison of mean PPD across three different time points suggested statistically significant differences with a p-value < 0.0001, at both the sites, using repeated measure ANOVA.

Mean CAL, for premolar, at 1 and 3 months post treatment for test site was (5.55±0.92 mm, 4.72±0.87 mm respectively) and for control site was (5.67±0.93 mm, 5.06±0.93 mm respectively). While for molar, mean CAL at 1 and 3 months post treatment for test site was (6.48±1.09 mm, 5.78±1.09 mm respectively) and for control site was (6.80±1.51 mm, 6±1.50 mm respectively). For premolar and molar, the comparison of mean CAL at baseline, 1 and 3 months suggested statistically significant differences with a p-value < 0.0001, at both the sites, using repeated measure ANOVA. Though gain in CAL was more in test site, CAL showed statistically insignificant mean difference between test site and control site at 3 different time points.

These results were similar to those reported by **Chondros P et al. (2009)[76]** in which effect of the adjunctive use of PDT in non-surgical periodontal treatment was assessed

in patients receiving supportive periodontal therapy where a diode laser of wavelength 670 nm along with a commercial solution based on a phenothiazine chloride dye was used and reported no statistically significant differences between the groups in terms of PPD and CAL at 3 and 6 months post-treatment. Similar results were reported by **Christodoulides N et al. (2008)[14], Theodoro LH et al. (2012)[35], Tabenski L et al. (2017)[41]. Christodoulides N et al. (2008)[14]** evaluated the clinical and microbiologic effects of the adjunctive use of PDT (performed with HELBO Blue Photosensitizer and 670 nm diode laser) to non-surgical periodontal treatment in chronic periodontitis and reported that both treatment modalities may lead to statistically significant improvements in all investigated clinical parameters at 3 and 6 months following therapy. However, no statistically significant difference in terms of CAL and PD changes was found between the two groups. Similar results were reported by **Theodoro LH et al. (2012)[35]** who evaluated the long-term clinical and microbiological effects of PDT associated with nonsurgical periodontal treatment by using TBO as photosensitizer and low level laser with wavelength of 660 nm in chronic periodontitis. **Tabenski L et al. (2017)[41]** investigated the additional influence of PDT vs. local application of minocycline microspheres following non-surgical periodontal therapy in deep periodontal pockets and showed that differences in clinical parameters between groups were not statistically significant.

However, contrasting results were shown in a study by **Campos GN et al. (2013)[34]** who found significantly higher probing pocket depth reduction and clinical attachment level gain in the PDT +SRP group at 3 months in residual pockets in single-rooted teeth. Contradictory results were also shown by **Betsy J et al. (2014)[36]** where they use methylene blue (MB) as a photosensitizer and diode laser of 655 nm in test group and

SRP alone in control group and reported statistically significant probing pocket depth reduction and clinical attachment level gain in test group as compared to control group.

The variation in the reported treatment outcome to PDT, in the literature, can be attributed to several factors such as drug ion concentration, period of retention of the drug within the tissue, mode of drug application, pH of the environment (tissue/tooth interface), presence of exudates and gingival fluid and time for biological response. A likely concern for the clinical application of PDT is the potential photo cytotoxicity to host cells. However, it has been revealed that the doses of light needed for killing bacteria in PDT are much lower than those that are toxic for fibroblasts and keratinocytes.[70]

Studies have showed that inflammatory cytokines, are produced during inflammatory responses have been accompanied with the onset or progression of tissue insult. In our study, reduction in GCF IL-6 levels from baseline to 3 months was observed. At baseline, mean GCF IL-6 level at test site was 8.64±0.64 pg/ml which was reduced significantly to 4.29±0.67 pg/ml at 3 months. While at control site mean GCF IL-6 level reduced significantly from 8.48±0.70 pg/ml at baseline to 5.83±0.65 pg/ml at 3 months. Mean GCF IL-6 level showed a statistically significant difference between test site and control site at 3 months, with a p-value < 0.0001. For both sites, mean GCF IL-6 level showed statistically significant difference at 3 months as indicated by p-value < 0.0001 using paired t-test**.** These findings are in accordance with the findings of **Luchesi VH et al. (2013)[28], Vohra F et al. (2018)[77], Reis C et al. (2014)[78]**. **Luchesi VH et al. (2013)[28]** investigated the effect of photodynamic therapy (PDT) as an adjunct to mechanical therapy in furcations. They evaluated clinical, microbiological and cytokine pattern at baseline, 3 and 6 months and reported that pro-inflammatory mediators GM-CSF, IL-8,

IL-1β, and IL-6 exhibited reduced levels in PDT group compared with control group, at 3 months post therapy. In this study GCF collection was done by filter paper strips and assesed by high sensitivity human cytokine 10-plex assay. **Vohra F et al. (2018)[77]** assessed the effect of antimicrobial photodynamic therapy (aPDT) as an adjunct to SRP on clinical, periodontal and immunological parameters in obese chronic periodontitis patients and showed that IL-6 and TNF-α levels decreased significantly at 12 weeks after therapy in both the groups and also intergroup comparison showed significant difference for aPDT group at 12 week follow-up. **Reis C et al. (2014)[78]** reported that nonsurgical periodontal therapy resulted in a statistically significant decrease in the total levels of IL-1α, IL-1β and IL-6 in the GCF of chronic periodontitis patients.

At baseline, mean GCF IL-8 level at test site was 456.53±9.42 pg/ml which was reduced significantly to 308.16±36.04 pg/ml at 3 months. While at control site mean GCF IL-8 level reduced significantly from 454.25±8.51 pg/ml at baseline to 379.51±7.32 pg/ml at 3 months. Mean GCF IL-8 level showed a statistically significant difference between test site and control site at 3 months, with a p-value < 0.0001. For both sites, mean GCF IL-8 level showed statistically significant difference at 3 months as indicated by p-value < 0.0001 using paired t-test**.**

These findings are similar to the findings of **Luchesi VH et al. (2013)[28], Gamonal J et al. (2000)[60], Mastromatteo-Alberga P et al. (2018)[24]**. **Luchesi VH et al. (2013)[28]** investigated the effect of photodynamic therapy (PDT) as an adjunct to mechanical therapy in furcations. They evaluated clinical, microbiological and cytokine pattern at baseline, 3 and 6 months and reported that pro-inflammatory mediators GM-CSF, IL-8, IL-1β, and IL-6 exhibited reduced levels in PDT group compared with control group, at 3 months post therapy. **Gamonal J et al. (2000)[60]** investigated the effect of periodontal

therapy on cytokine levels in GCF where GCF samples were collected from active and inactive sites with a paper strip and assessed via. ELISA kits and reported that total amount of IL-1β, IL-8, IL-10 and RANTES reduced by periodontal therapy. **Mastromatteo-Alberga P et al. (2018)[24]** determined the levels of IL-1α, IL-1β, TNF-α, IL-6, IL-6sR, IL-8, IL-10, MMP- 3 and MMP-8 in GCF, before and after NSPT where they used paper points for collection of GCF and measured by ELISA and reported that all inflammatory mediators decreased after therapy; IL-6, Il-6sR, IL-10 and TNF-α, attained the highest reduction (70% - 54%); the values of MMP3, IL-1α, IL-1β and IL-8 were reduced between 50% - 34%; and MMP-8 showed the lowest decrease (28%).

These findings are in line with the present study, supporting the immunomodulatory performance of PDT. Accordingly, formerly reported data have already specified the capacity of PDT to modulate the host response because of its ability to reduce the stimulation of T lymphocytes, interfere with the immunostimulatory function of antigen-presenting cells and inactivate pro-inflammatory mediators.[30]

Mainly by downregulating the production of proinflammatory cytokines and stimulating protective antibody production, IL-10 is supposed to play a role in periodontitis. In our study, at baseline, mean GCF IL-10 level at test site was 11.77$\pm$0.75 pg/ml which was increased significantly to 14.25$\pm$0.83 pg/ml at 3 months. While at control site mean GCF IL-10 level increased significantly from 11.56$\pm$0.73 pg/ml at baseline to 12.48$\pm$0.82 pg/ml at 3 months. Mean GCF IL-10 level showed a statistically significant difference between test site and control site at 3 months, with a p-value < 0.0001. For both sites, mean GCF IL-10 level showed statistically significant difference at 3 months as indicated by p-value < 0.0001 using paired t-test**.**

These findings are similar to the findings of **da Cruz Andrade PS et al. (2017)[79]** who evaluated the effect of PDT on inflammatory mediator levels in residual periodontal pockets of patients with severe chronic periodontitis under periodontal maintenance, during 12 months follow-up where after SRP, 0.01% methylene blue and 660nm diode laser was used in test group while saline irrigation and laser fibre device without activation was used in control group and reported that photodynamic therapy promoted an increase in IL-10 levels at 3 months compared to the baseline and at 7 days, and this increase was not observed in the control group. Similar trend was also observed in a study by **Costa FO et al. (2018)[62]** among regular compliers (RC) and irregular compliers (IC) during periodontal maintenance therapy (PMT) where the authors found that Levels of IL-10 increased among RC and reduced among IC. On the contrary, **de Melo Soares MS et al. (2019)[33]** mentioned that multiple applications of antimicrobial photodynamic therapy (aPDT) in smokers with chronic periodontitis did not improve significantly the clinical, immunological, and microbiological parameters when compared with SRP alone where authors found comparable levels of Levels of anti-inflammatory cytokines and bacterial species after 90 days. Authors explained that the impairment of the host immune system by substances in cigarette smoke leads to changes in polymorphonuclear leukocyte function such as chemotaxis, phagocytosis, and oxidative burst.

Conflicting results shown in a study by **Kolbe MF et al. (2014)[30]** where they investigated the effect of photodynamic therapy (PDT) as monotherapy during supportive periodontal therapy along with evaluation of clinical, microbiologic (real-time polymerase chain reaction analyses), cytokine pattern (multiplexed bead immunoassay), and patient-centered outcome (regarding morbidity) performed at

baseline, 3 months and 6 months in PDT group (methylene blue + diode laser with a wavelength of 660 nm), PS group (methylene blue) and SRP group and reported that patients in the PDT protocol exhibited augmented levels of anti-inflammatory IL-4 and IL-10 and reduced pro-inflammatory IL-1β and IL-6 at 6 months. IL-8 level reduced at 3 months and increased at 6 months in PDT group while in SRP group IL-8 level increased at 3 months and reduced at 6 months but differences were not statistically significant.

PDT is a recognized non-antibiotic bactericidal approach. The bactericidal effect of aPDT is based upon the preferential binding of the photosensitizer, in our study, toluidine blue O, to the bacterial cell surface. The photosensitizer molecule absorbs light and generates highly reactive O_2 species (singlet O_2), which can damage a wide variety of proteins, carbohydrates and lipids. As singlet O_2 has a very short half-life and, therefore, its destructive radius is small, resulting in photoreactive effects only in very close proximity to where the photosensitizer compound is located. This localized reaction, and the fact that bacterial cells are not armed with the same ability to scavenge reactive oxygen compounds such as eukaryotic cells, makes PDT highly bactericidal with little damage to the surrounding tissue. PDT treatment has the potential to inhibit destructive host responses by killing periopathogenic bacteria, this may contribute to its clinical usefulness as an adjunctive therapy.[80] The role of photodynamic therapy on the levels of the inflammatory mediators are described by some studies, however, it is essential to highlight that the clinical conditions such as time of performance and tissue photosensitizer concentration, exudate presence, pH change and gingival fluid in the subgingival environment can influence the effectiveness of therapy.

The literature suggests that the effect of PDT as an adjunct to NSPT and NSPT alone have beneficial effect in controlling periodontal inflammation thus improving clinical and biochemical parameters in periodontitis patients. However, this study plays a crucial role in demonstrating the effect of PDT as an adjunct to NSPT and NSPT alone in reduction of periodontal inflammation in patients with chronic periodontitis and also in evaluating the GCF levels of IL-6, IL-8 and IL-10 at both sites and their levels after PDT and NSPT.

Limitations

There are few limitations of the study:

1. In this study two applications of PDT was performed which could have affected the clinical outcome. Future studies are needed to ultimately explain to what extent multiple applications of PDT might improve the outcome of therapy. There is a lack of an established protocol for PDT with NSPT. Further studies are therefore needed to determine a more effective treatment protocol by using PDT as an adjunct to nonsurgical treatment of periodontitis.

2. In this study the split-mouth design was used. Periodontal therapy focuses on reducing the levels of periodontopathogens, but during the healing period intraoral transmission of periodontopathogens occurs which may have affected treatment outcome.

3. A larger sample size with long term observation period is desirable for substantiation of the findings.

4. The operator was the assessor in the present study and there were no blinded examinations. Therefore possibility of operator bias to some extent cannot be ruled out.

Conclusion

The present randomized controlled study was undertaken to evaluate the efficacy of PDT as an adjunct to NSPT and NSPT alone on the GCF levels of IL-6, IL-8 & IL-10 in chronic periodontitis. 21 systemically healthy patients with moderate to severe chronic periodontitis were recruited for the study. Two contralateral sites (premolar and molar) per patient were randomly assigned to test site (treated with PDT + NSPT) and control site (NSPT alone). Clinical parameters evaluated were PI, GI, BOP, PPD and CAL at baseline, 1 and 3 months after therapy. Biochemical parameters including GCF levels of IL-6, IL-8 and IL-10 were analysed by ELISA test at baseline and 3 months after therapy.

During the course of the study, there was no untoward allergic reaction indicating the biocompatibility of the material used. Throughout the study period, declines in PI and GI indicated satisfactory maintenance of oral hygiene by the patients. A statistically greater BOP reduction in test sites than in control sites at 1 and 3 months post therapy specified reduction in periodontal inflammatory symptoms. A statistically significant improvement in PPD and CAL of both sites at 1 and 3 months indicated that both PDT as an adjunct to NSPT and NSPT alone was effective. Also, there was statistically significant reduction in GCF levels of IL-6 and IL-8 while there was a significant increase in IL-10 levels at test sites than in control sites at 3 months. This pointed to the effectiveness of PDT as an adjunct to NSPT in improvement of biochemical parameters.

From the analysis of the results, following observations can be drawn:

1. PDT + NSPT resulted in statistically significant reductions in GCF levels of IL-6 and IL-8 at 3 months, compared to NSPT alone.
2. PDT + NSPT resulted in statistically significant increase in GCF levels of IL-10 at 3 months, compared to NSPT alone.
3. PDT + NSPT resulted in statistically significant reductions in BOP at 1 and 3 months, compared to NSPT alone.
4. Both PDT + NSPT and NSPT alone resulted in statistically significant reductions in PPD and CAL at 1 and 3 months.

Within the limitations of the study it can be concluded that, applications of two sessions of PDT, adjunctive to NSPT, resulted in reduction in GCF levels of pro- inflammatory cytokines and increase in levels of anti-inflammatory cytokines in chronic periodontitis patients. Also, adjunctive treatment modality promoted significant reduction in BOP, signifying a possible positive effect on periodontal healing. However, both treatment modalities showed comparable results in terms of PPD reduction and CAL gain. Considering the safety, the lack of side effects and general advantages like more patient compliance, the PDT treatment with NSPT is recommended as an efficient adjunctive modality for the treatment of chronic periodontitis.

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Tables

Table 1: **Descriptive statistics for demographic parameters of patients**

Parameters	Value
Age (year)[Mean ± SD]	44.95±8.51
Sex [No. (%)]	
Male	7 (33.34)
Female	14 (66.66)

Table 2: Comparison of clinical parameter for different time intervals

Parameters	Baseline (n=21)	1 month (n=21)	3 month (n=21)	P-value*
Plaque Index	2.39±0.51	1.53±0.53	0.97±0.42	< **0.0001 (S)**
Gingival Index	2.09±0.59	1.20±0.38	0.98±0.43	< **0.0001 (S)**

*Obtained using repeated measures of ANOVA; S: Significant

Table 3: Comparison of BOP between two sites and across time at each site.

BOP	Test site (n=21)		Control site (n=21)		P-value*
	Mean	SD	Mean	SD	
Baseline	64.10	7.67	64.43	7.58	0.888 (NS)
1 month	41.10	3.58	45.29	3.90	**0.001 (S)**
3 month	38.00	3.62	46.81	4.33	**< 0.0001 (S)**
P-value[Ŧ]	**< 0.0001 (S)**		**< 0.0001 (S)**		

*Obtained using paired t-test; [Ŧ] Obtained using repeated measures of ANOVA;
S: Significant, BOP: bleeding on probing

Table 4: Comparison of PPD between two sites and across time at each site

PPD	Test site (n=21)		Control site (n=21)		P-value*
	Mean	SD	Mean	SD	
Premolar					
Baseline	5.23	0.65	5.14	0.70	0.444 (NS)
1 month	4.10	0.64	4.37	0.69	**0.021 (S)**
3 month	3.42	0.63	3.66	0.66	**0.022 (S)**
P-value [Ŧ]	**< 0.0001 (S)**		**< 0.0001 (S)**		
Molar					
Baseline	5.92	0.71	5.78	0.54	0.134 (NS)
1 month	4.82	0.71	4.80	0.67	0.836 (NS)
3 month	4.12	0.71	4.15	0.68	0.722 (NS)
P-value [Ŧ]	**< 0.0001 (S)**		**< 0.0001 (S)**		

*Obtained using paired t-test; [Ŧ] Obtained using repeated measures of ANOVA;
S: Significant; NS: Not Significant, PPD: Probing pocket depth

Table 5: Comparison of CAL between two sites and across time for each site

CAL	Test site (n=21)		Control site (n=21)		P-value*
	Mean	SD	Mean	SD	
Premolar					
Baseline	6.67	0.91	6.46	0.93	0.319 (NS)
1 month	5.55	0.92	5.67	0.93	0.570 (NS)
3 month	4.72	0.87	5.06	0.93	0.088 (NS)
P-value ₸	< **0.0001 (S)**		< **0.0001 (S)**		
Molar					
Baseline	7.58	1.09	7.52	1.47	0.790 (NS)
1 month	6.48	1.09	6.80	1.51	0.171 (NS)
3 month	5.78	1.09	6.00	1.50	0.345 (NS)
P-value ₸	< **0.0001 (S)**		< **0.0001 (S)**		

*Obtained using paired t-test; ₸ Obtained using repeated measures of ANOVA;
S: Significant; NS: Not Significant, CAL: clinical attachment level

Table 6: Comparison of IL-6, IL-8 and IL-10 between two sites and across times

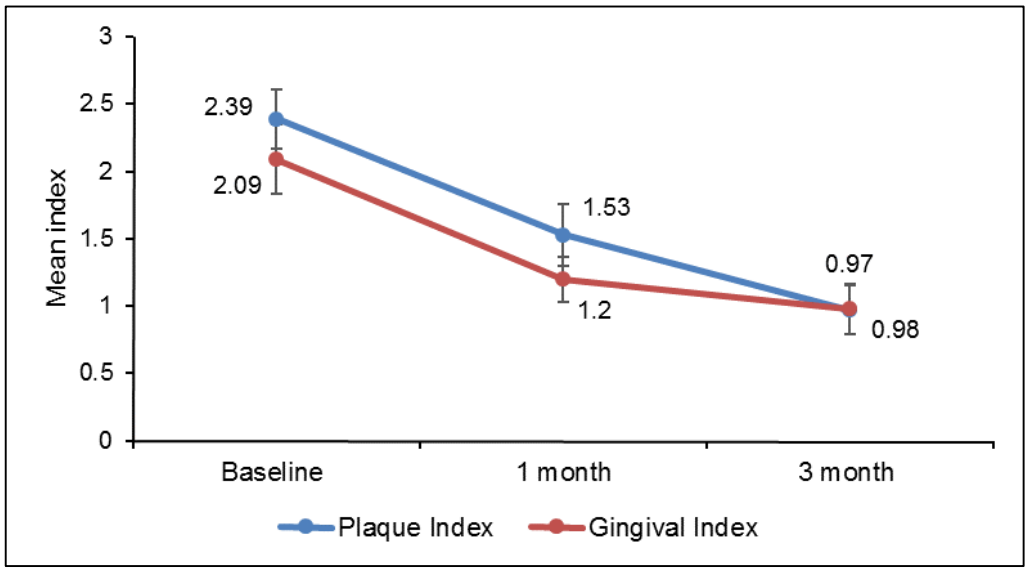
	Test site (n=21)		Control site (n=21)		P-value*
	Mean	SD	Mean	SD	
IL 6 (pg/ml)					
Baseline	8.65	0.64	8.48	0.70	0.087 (NS)
3 month	4.29	0.67	5.83	0.65	< **0.0001 (S)**
P-value*	< **0.0001 (S)**		< **0.0001 (S)**		
IL 8 (pg/ml)					
Baseline	456.53	9.42	454.25	8.51	0.161 (NS)
3 month	308.16	36.04	379.51	7.32	< **0.0001 (S)**
P-value*	< **0.0001 (S)**		< **0.0001 (S)**		
IL 10 (pg/ml)					
Baseline	11.77	0.75	11.56	0.73	0.141 (NS)
3 month	14.25	0.83	12.48	0.82	< **0.0001 (S)**
P-value*	< **0.0001 (S)**		< **0.0001 (S)**		

*Obtained using paired t-test; S: Significant; NS: Not Significant, IL-6: Interleukin-6, IL-8: Interleukin-8, IL-10: Interleukin-10

Graphs

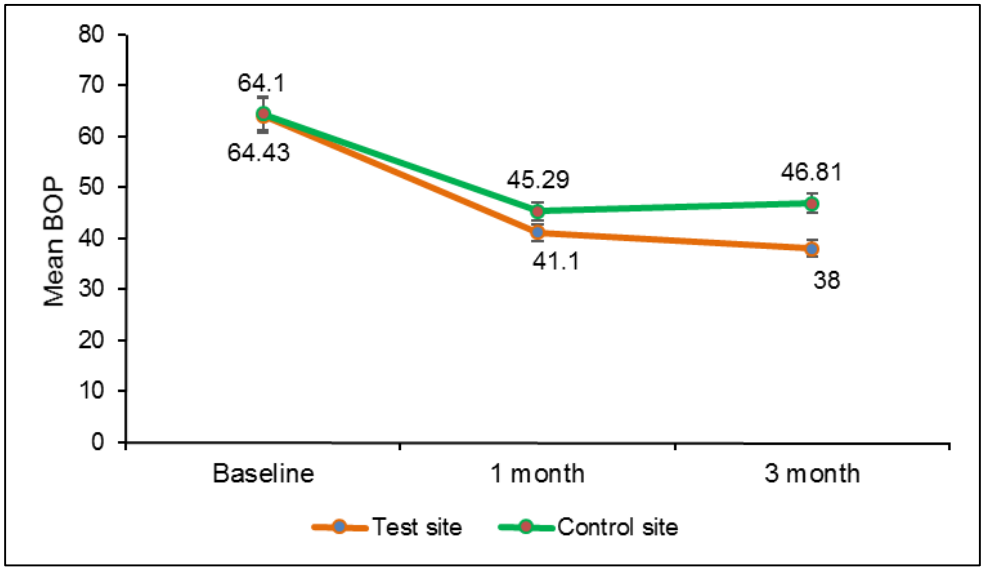
Graph 1

Line chart showing mean plaque and gingival index values at different



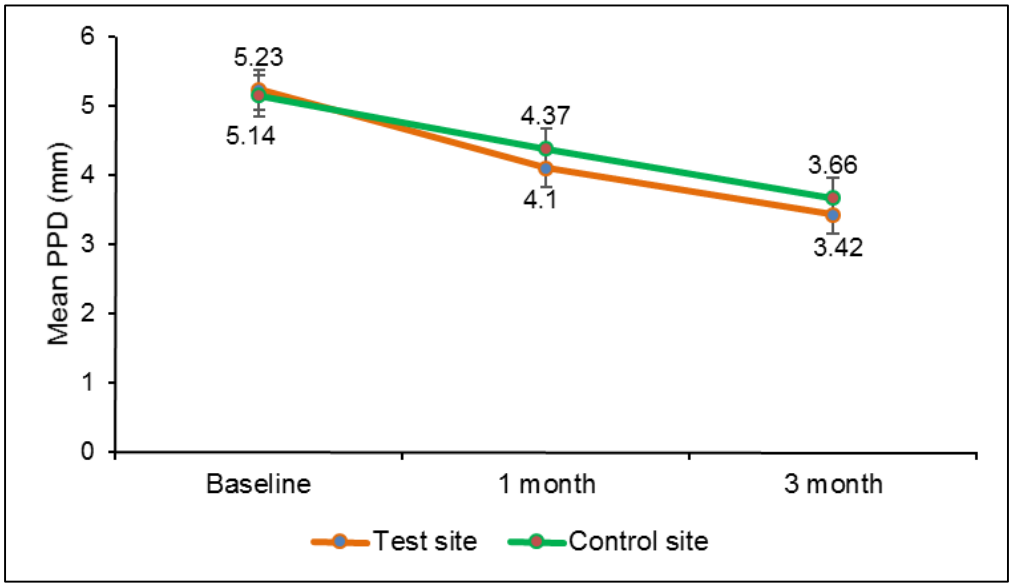
Graph 2

Line chart showing mean BOP at different times for two study sites



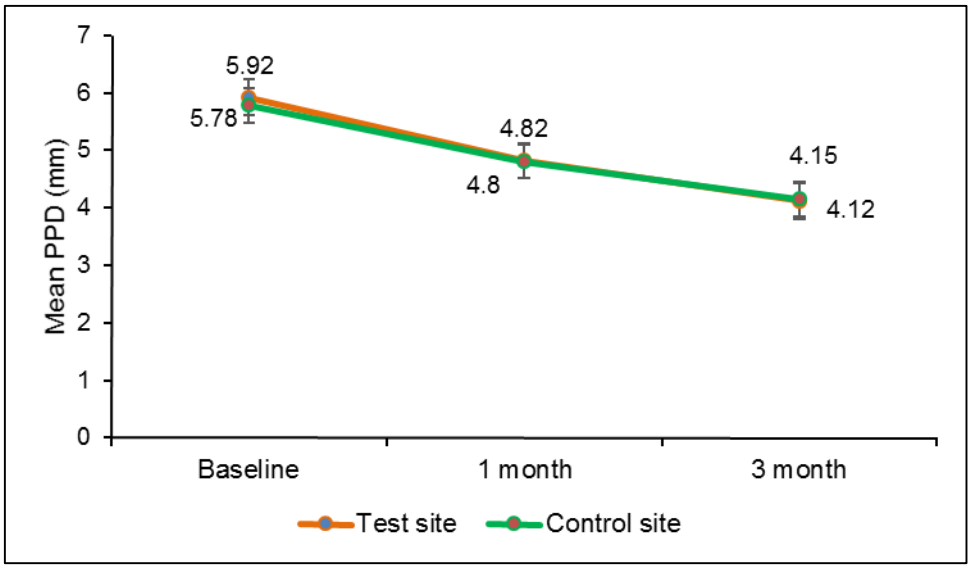
Graph 3

Line chart showing mean PPD at different times for two study sites for premolar



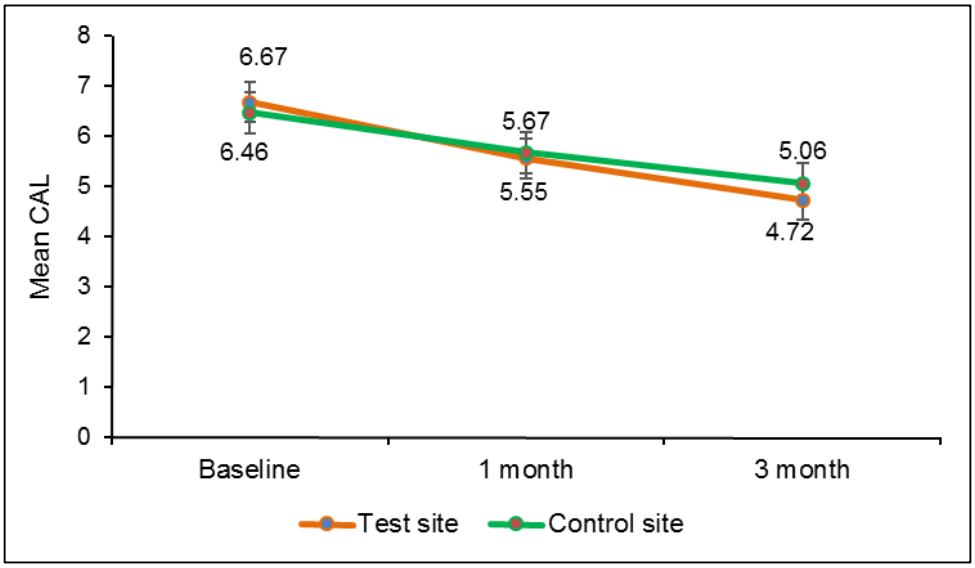
Graph 4

Line chart showing mean PPD at different times for two study sites for molar



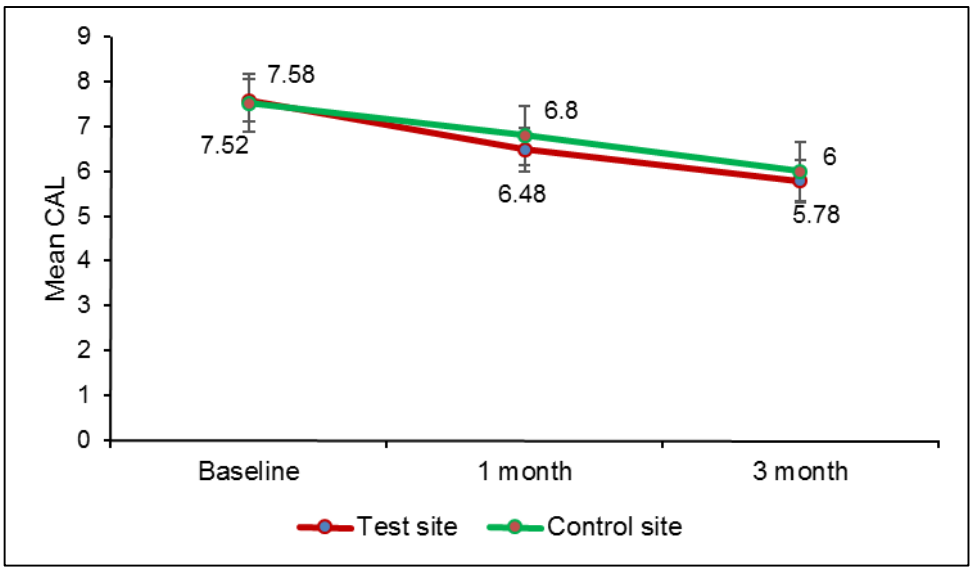
Graph 5

Line chart showing mean CAL at different times for two study sites for premolar



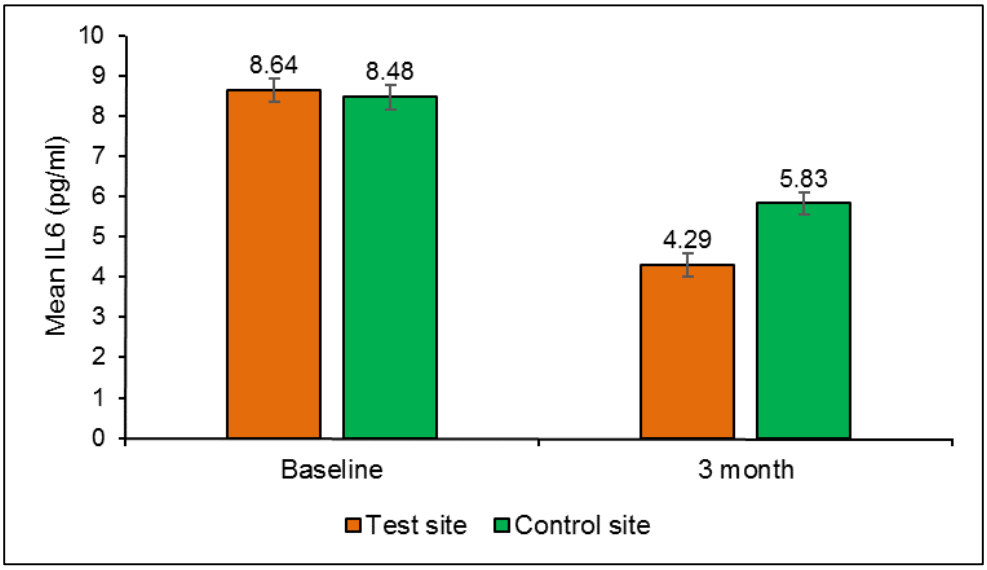
Graph 6

Line chart showing mean CAL at different times for two study sites for molar



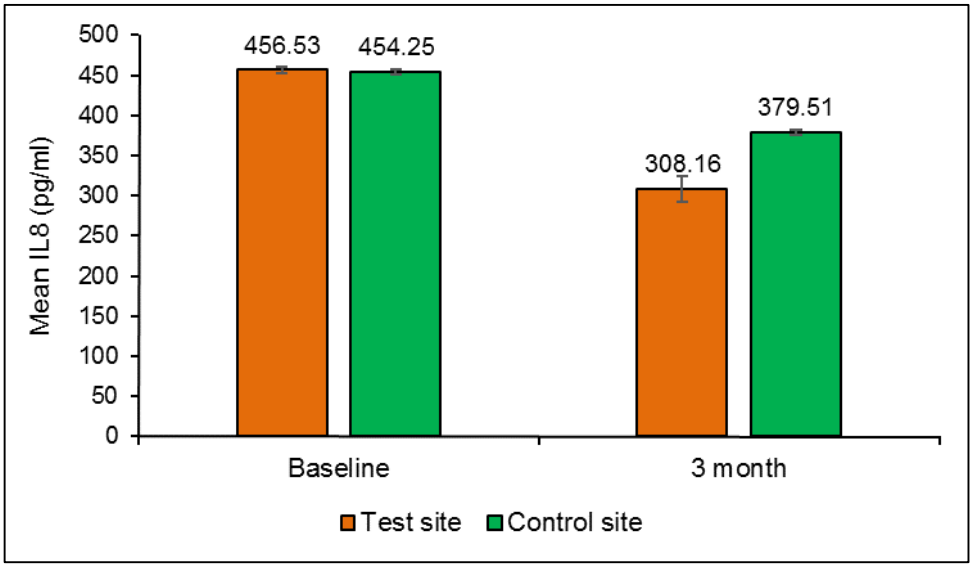
Graph 7

Column chart showing mean IL-6 at different times for two study sites



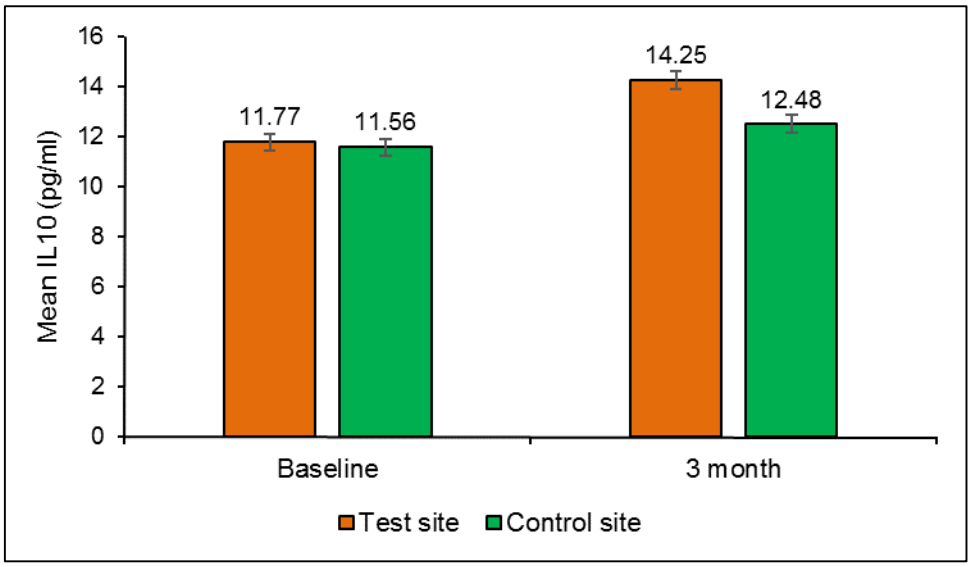
Graph 8

Column chart showing mean IL-8 at different times for two study sites



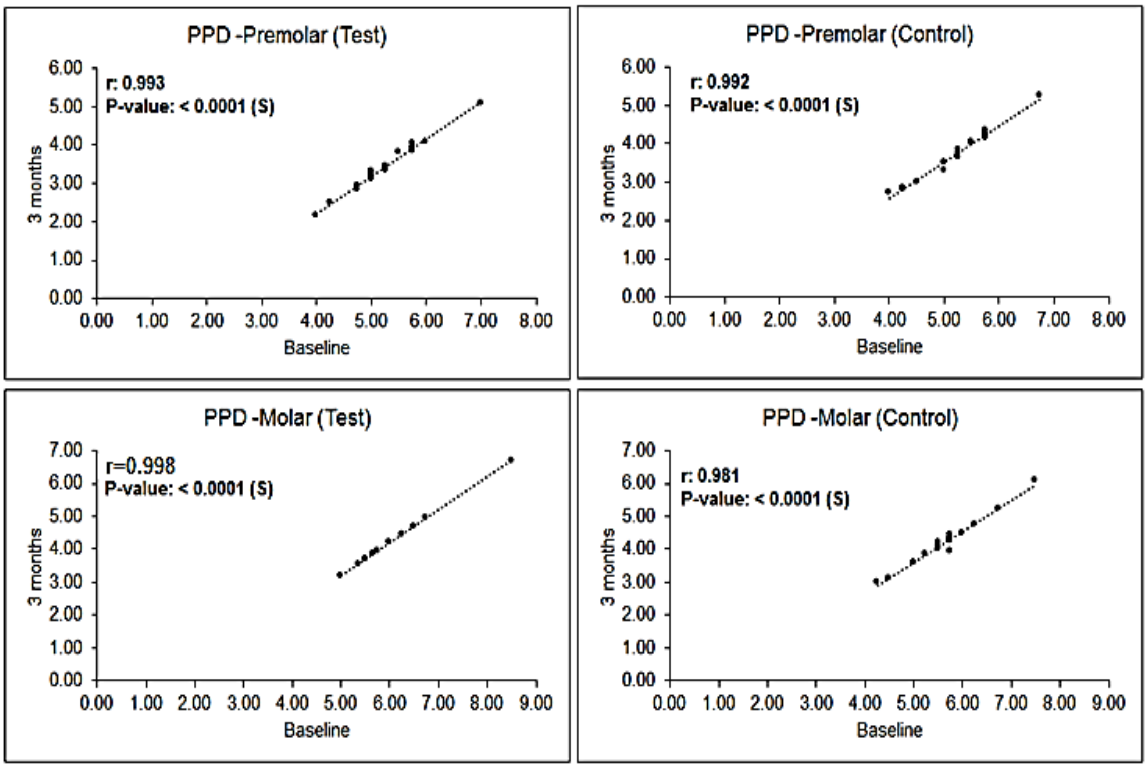
Graph 9

Column chart showing mean IL-10 at different times for two study sites



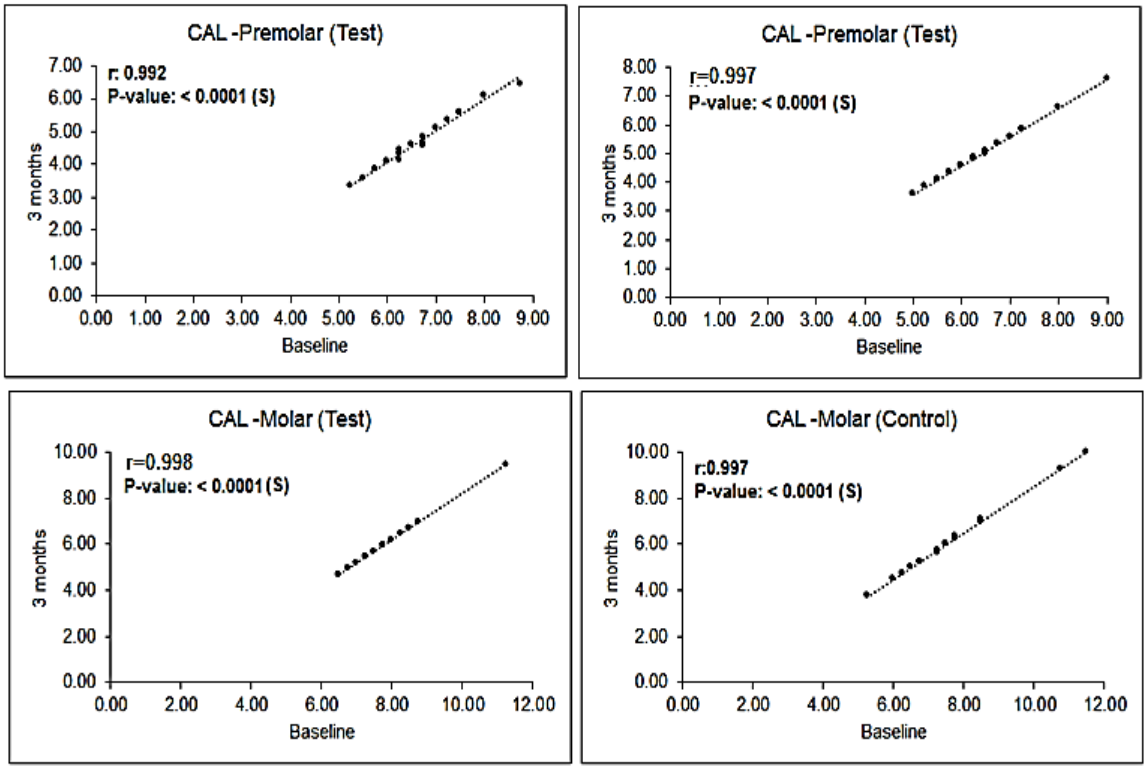
` Graph 10

Scatter plot showing correlation of PPD between baseline and 3 months for molar and premolar teeth at test and control sites



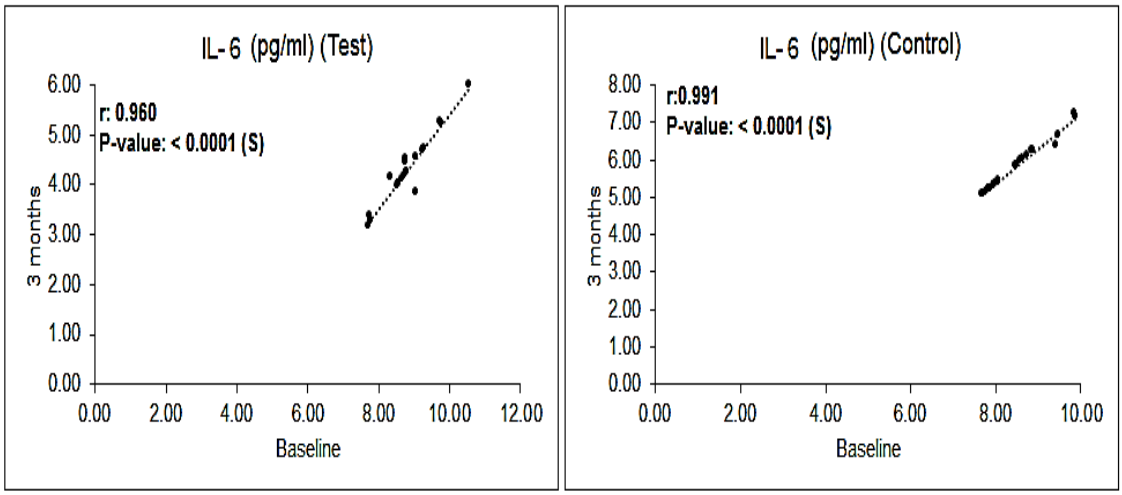
Graph 11

Scatter plot showing correlation of CAL between baseline and 3 months for molar and premolar teeth at test and control sites



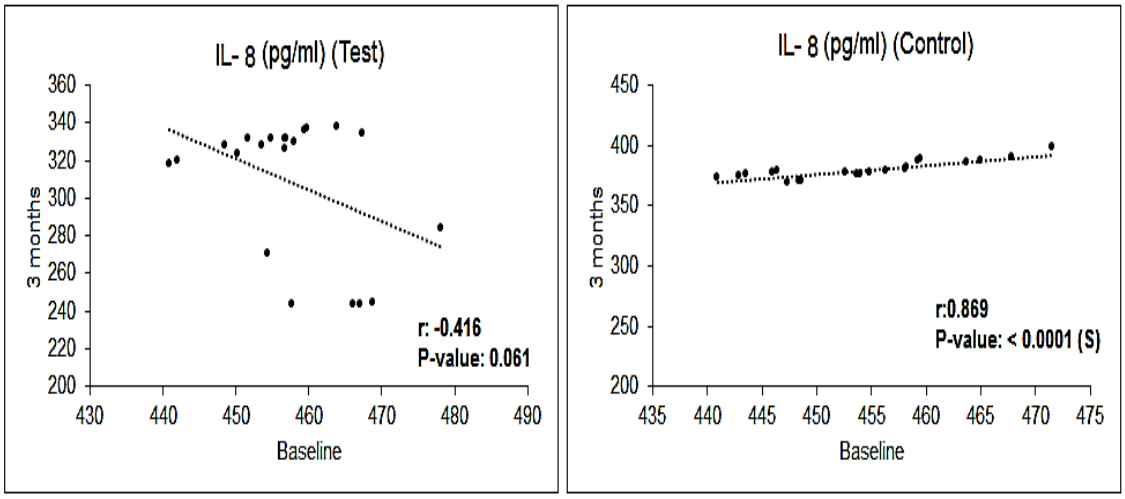
Graph 12

Scatter plot showing correlation of IL-6 between baseline and 3 months at test and control sites



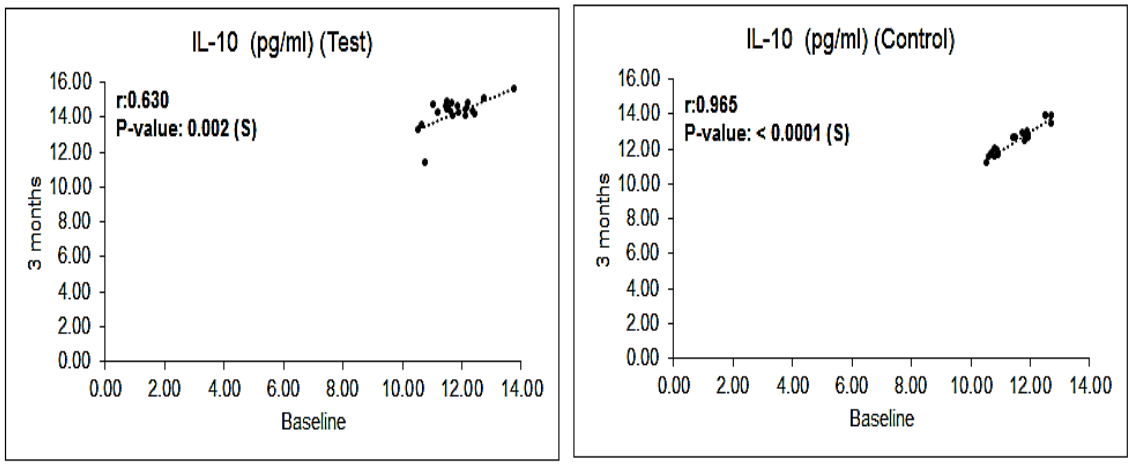
Graph 13

Scatter plot showing correlation of IL-8 between baseline and 3 months at test and control sites



Graph 14

Scatter plot showing correlation of IL-10 between baseline and 3 months at test and control sites



Master chart

Demographic characteristics

Sr. No	Age	Gender
1	47	M
2	44	M
3	50	F
4	36	F
5	36	F
6	48	M
7	45	F
8	37	F
9	40	M
10	35	M
11	38	F
12	53	F
13	38	M
14	35	F
15	65	F
16	51	M
17	55	F
18	48	F
19	57	F
20	36	F
21	50	F

CLINICAL PARAMETERS

Plaque Index (PI) and Gingival Index (GI)

Sr. No	PI			GI		
	Baseline	**1 month**	**3 months**	**Baseline**	**1 month**	**3 months**
1	2.75	1.45	1.2	1.45	0.833	0.6
2	1.062	0.916	0.75	1.52	0.55	0.7
3	2.75	1.58	1.45	2.41	1.79	1.58
4	2.66	0.833	0.7	1.45	1.45	0.85
5	1.75	0.55	0.54	2.62	0.916	1.61
6	1.62	1.29	0.58	1.45	0.73	0.5
7	2.66	1.33	0.54	1.45	0.66	0.51
8	1.87	1.25	0.75	2.04	0.87	0.54
9	2.75	1.12	0.5	1.45	0.7	0.5
10	2.75	2	0.75	2.41	1.37	0.68
11	2.58	1.61	1.29	2.83	1.29	1.83
12	2.58	1.87	0.95	3	1.37	0.95
13	2.58	3	2.2	3	1.12	0.78
14	1.58	1.2	0.66	1.91	1.45	0.85
15	2.75	1.66	0.75	2.41	1.41	0.97
16	2.75	1.58	1.25	2.41	1.2	1.1
17	2.33	1.37	0.95	1.57	1.19	0.78
18	2.75	1.79	0.75	2.79	1.75	1.87
19	2.75	2.29	1.45	2.54	1.66	1.22
20	2.75	1.87	1.33	1.24	1.62	1.18
21	2.2	1.5	1	1.91	1.37	1.08

CLINICAL PARAMETERS

Bleeding on Probing (BOP) at Test sites and Control sites

Sr. No	**BOP- test site** (%)			**BOP-control site** (%)		
	Baseline	**1 month**	**3 months**	**Baseline**	**1 month**	**3 months**
1	54	36	33	54	40	42
2	59	42	39	62	45	47
3	68	44	41	67	49	51
4	56	37	34	54	41	42
5	70	44	41	68	49	51
6	53	36	33	54	40	39
7	55	34	31	54	38	40
8	64	42	39	65	46	48
9	54	37	33	53	40	42
10	70	43	40	71	48	51
11	72	45	42	73	49	51
12	75	46	43	74	51	53
13	76	47	44	75	52	54
14	59	41	38	61	44	42
15	68	43	40	69	47	49
16	69	43	39	68	47	47
17	58	40	37	64	44	46
18	73	44	41	74	48	49
19	71	40	37	72	45	47
20	58	39	36	58	43	45
21	64	40	37	63	45	47

CLINICAL PARAMETERS

Probing Pocket Depth (PPD) at Test sites and Control sites

Sr. No	Test site- PPD						Control site- PPD					
	Baseline		1 month		3 months		Baseline		1 month		3 months	
	P	M	P	M	P	M	P	M	P	M	P	M
1	5.5	5.75	4.5	4.65	3.8	3.95	5.75	6.25	4.95	5.45	4.25	4.75
2	7	8.5	5.8	7.4	5.1	6.7	6.75	7.5	5.85	6.7	5.25	6.1
3	5.25	5.5	4.15	4.4	3.45	3.7	5.75	5.5	4.95	4.7	4.35	4.1
4	5.75	6.75	4.55	5.65	3.85	4.95	5.75	6.75	5.05	5.95	4.25	5.25
5	5.75	6.25	4.75	5.15	4.05	4.45	5.5	6	4.7	5.2	4.05	4.5
6	5.25	5.75	4.1	4.65	3.45	3.95	5.25	5.25	4.55	4.55	3.75	3.85
7	5.25	5.5	4.05	4.4	3.35	3.7	4.25	6	3.45	4.3	2.85	3.6
8	5.75	6	4.65	4.9	3.95	4.2	5.25	5.75	4.45	4.95	3.85	4.35
9	5	5.75	3.9	4.65	3.2	3.95	5	5.5	4.3	4.7	3.5	4
10	5	6.25	4	5.15	3.3	4.45	5.5	6	4.8	4.95	4	4.25
11	5	5.65	3.8	4.55	3.1	3.85	4.5	5	3.8	4.2	3	3.6
12	4.75	5.75	3.65	4.65	2.95	3.95	4.25	5.75	3.45	4.2	2.85	3.6
13	4.25	5.5	3.1	4.4	2.5	3.7	5	5.5	4.3	4.7	3.5	4.1
14	5.75	5.75	4.6	4.65	3.9	3.95	5.25	5.75	4.5	4.85	3.75	4.35
15	4	5.35	2.85	4.25	2.15	3.55	4	5.25	3.25	3.8	2.75	3.1
16	5	5.75	3.9	4.65	3.3	3.95	4.25	5.5	3.45	4.7	2.85	4.1
17	4.75	5.5	3.55	4.4	2.85	3.7	4.25	5.5	3.45	3.7	2.8	3
18	5	5.75	3.9	4.65	3.2	3.95	5.75	5.75	5.05	4.65	4.25	3.95
19	6	6.5	4.8	5.4	4.1	4.7	5.75	5.75	4.85	5.05	4.15	4.45
20	4.75	5	3.65	3.9	2.95	3.2	5.25	5.5	4.5	4.8	3.65	4.2
21	5	5.75	3.9	4.65	3.3	3.95	5	5.75	4.2	4.65	3.3	3.95

CLINICAL PARAMETERS

Clinical Attachment Level (CAL) at Test sites and Control sites

Sr. No	Test site- CAL						Control site- CAL					
	Baseline		1 month		3 months		Baseline		1 month		3 months	
	P	M	P	M	P	M	P	M	P	M	P	M
1	6.25	6.5	6.05	5.4	4.15	4.7	6.5	7.5	5.7	6.8	5	6
2	8	11.25	6.9	10.15	6.1	9.45	8	10.75	7.2	10.05	6.6	9.25
3	6.25	7.25	5.05	6.15	4.35	5.45	7.25	8.5	6.45	7.8	5.85	7
4	7	8.5	5.9	7.4	5.1	6.7	6.25	8.5	5.45	7.9	4.85	7.1
5	8.75	7.25	7.55	6.15	6.45	5.45	6.25	7.5	5.5	6.8	4.8	6
6	6.75	7.5	5.65	6.4	4.55	5.7	6	6.75	5.2	6.05	4.6	5.25
7	6.5	7.75	5.4	6.65	4.6	5.95	5.5	7.25	4.7	6.55	4.1	5.75
8	6.75	7.25	5.55	6.05	4.65	5.45	6.25	7.25	5.45	6.7	4.85	5.6
9	7.5	8.5	6.3	7.4	5.6	6.7	9	11.5	8.2	10.8	7.6	10
10	6.25	8.25	5.15	7.15	4.35	6.45	6.5	7.75	5.7	7.05	5.1	6.25
11	6	6.75	4.8	5.65	4.1	4.95	5	5.25	4.2	4.55	3.6	3.75
12	5.75	7.25	4.65	6.15	3.85	5.45	6.75	6	5.95	5.3	5.35	4.5
13	5.5	6.5	4.3	5.4	3.6	4.7	5.5	6.25	4.7	5.5	4.1	4.75
14	6.75	7.75	5.55	6.65	4.85	5.95	6.25	7.25	5.45	6.55	4.85	5.75
15	5.25	6.75	4.05	5.65	3.36	4.95	5.75	6.75	4.95	6.05	4.35	5.25
16	6.25	7	5.06	5.9	4.45	5.2	5.25	6.25	4.45	5.55	3.85	4.75
17	6	7.25	4.8	6.15	4.1	5.45	6	6.5	5.2	5.2	4.6	4.5
18	7.5	6.75	6.4	5.65	5.6	4.95	7	7.75	6.2	6.95	5.6	6.35
19	8	8.75	6.8	7.65	6.1	6.95	7	7.75	6.2	7.05	5.6	6.25
20	5.75	6.5	4.55	5.4	3.85	4.7	6.5	6.5	5.7	5.8	5.1	5
21	7.25	8	6.15	6.9	5.35	6.2	7.25	8.5	6.45	7.8	5.85	7

BIOCHEMICAL PARAMETERS

GCF IL-6 concentration at Test site and Control site

Sr. No	**IL-6** (pg/ml)			
	Test site IL-6		**Control site IL-6**	
	Baseline	**3 months**	**Baseline**	**3 months**
1	8.79	4.25	9.48	6.65
2	9.7	6	9.89	7.16
3	8.57	4.03	8.64	6.04
4	9.76	5.22	9.85	7.23
5	9.24	4.7	9.41	6.38
6	8.79	4.25	8.46	5.86
7	8.56	4.02	8.05	5.42
8	9.05	4.55	8.87	6.26
9	7.75	3.37	7.76	5.13
10	8.7	4.73	8.85	6.24
11	8.65	4.13	8.07	5.44
12	8.76	4.51	8.49	5.86
13	7.7	4.03	7.85	5.22
14	8.74	4.2	8.58	5.98
15	8.35	4.16	7.86	5.23
16	8	4.46	7.95	5.31
17	8.2	3.98	7.68	5.1
18	7.79	3.27	7.83	5.2
19	9.75	5.25	8.75	6.12
20	9.05	3.85	7.98	5.35
21	7.72	3.18	7.85	5.22

BIOCHEMICAL PARAMETERS

GCF IL-8 concentration at Test site and Control site

Sr.No	IL-8 (pg/ml)			
	Test site IL-8		Control site IL-8	
	Baseline	3 months	Baseline	3 months
1	461.52	243.48	465.05	387.24
2	478.16	283.77	471.57	397.84
3	454.34	270.51	458.18	380.45
4	463.96	337.59	467.86	389.84
5	467.36	334.45	463.77	385.64
6	456.76	325.58	456.37	378.33
7	451.63	331.6	452.65	377.54
8	452.12	243.53	459.26	386.95
9	448.6	327.69	440.94	372.74
10	468.84	243.84	454.06	376.35
11	459.86	336.93	458.21	380.84
12	456.96	331.66	453.74	375.45
13	453.65	328.15	447.36	369.04
14	450.35	323.65	459.49	387.95
15	442.12	329.76	445.96	377.24
16	454.85	331.36	443.58	375.65
17	459.45	335.45	446.35	378.36
18	441.95	319.87	448.67	370.77
19	467.15	243.75	454.85	376.85
20	456.77	331.07	448.43	370.17
21	440.84	317.68	442.88	374.56

BIOCHEMICAL PARAMETERS

GCF IL-10 concentration at Test site and Control site

Sr.No	**IL-10** (pg/ml)			
	Test site IL-10		**Control site IL-10**	
	Baseline	**3 months**	**Baseline**	**3 months**
1	11.54	14.81	12.53	13.85
2	13.79	15.58	12.74	13.89
3	11.59	14.52	11.55	12.57
4	12.23	14.72	12.73	13.38
5	12.78	14.98	12.53	13.86
6	11.05	14.64	11.45	12.59
7	11.5	14.61	11.87	12.68
8	12.17	14.02	11.76	12.85
9	10.81	11.3	10.64	11.56
10	12.46	14.15	11.94	12.94
11	11.65	14.31	11.84	12.7
12	11.53	14.37	11.96	12.58
13	11.69	14.73	10.71	11.67
14	11.87	14.55	11.96	12.78
15	11.21	14.2	10.83	11.5
16	11.92	14.25	10.93	11.64
17	11.72	14.07	10.55	11.14
18	10.57	13.23	10.71	11.58
19	12.38	14.3	11.83	12.46
20	12.15	14.37	10.84	11.98
21	10.66	13.52	10.92	11.84

Effect of Photodynamic therapy as an adjunct to Non-Surgical Periodontal therapy on Gingival Crevicular Fluid Interleukin-6, Interleukin-8 & Interleukin-10 levels in chronic periodontitis: A Randomized Controlled Trial

CASE HISTORY PROFORMA

NAME: **OPD NO:**

AGE/SEX: **DATE:**

ADDRESS: **PHONE NO:**

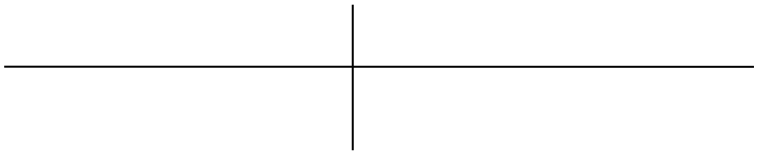
OCCUPATION:

CHIEF COMPLAINT:

PAST DENTAL HISTORY:

PAST MEDICAL HISTORY:

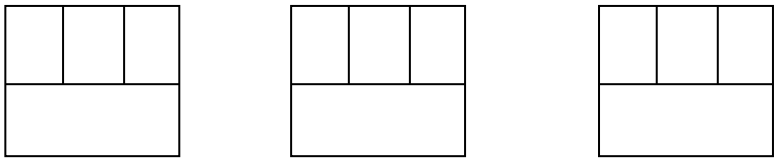
TEETH PRESENT:

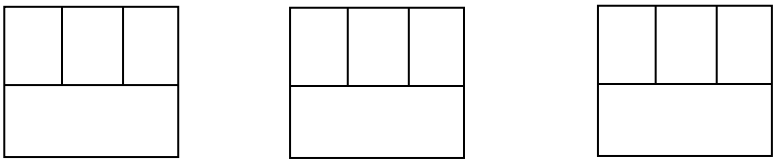


ORAL HYGIENE HABITS:

PLAQUE INDEX by Silness and Loe 1964 *(*At Baseline)

16 12 24





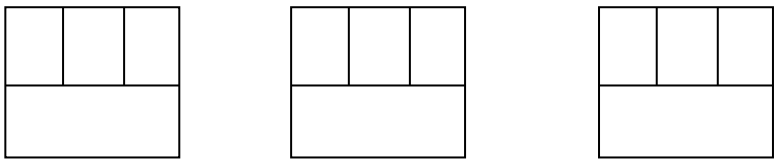
44 32 36

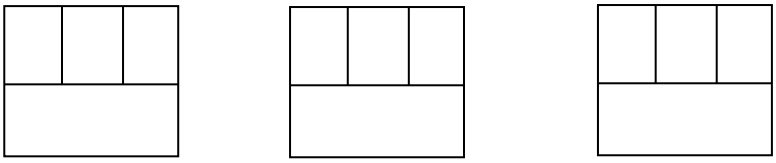
$$\frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$$

SCORE:

PLAQUE INDEX (After 1 month)

16 12 24



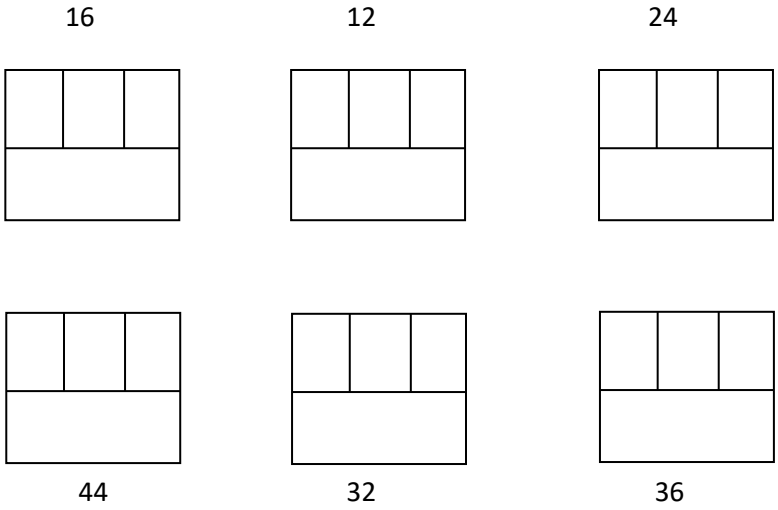


44 32 36

$$\frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$$

SCORE:

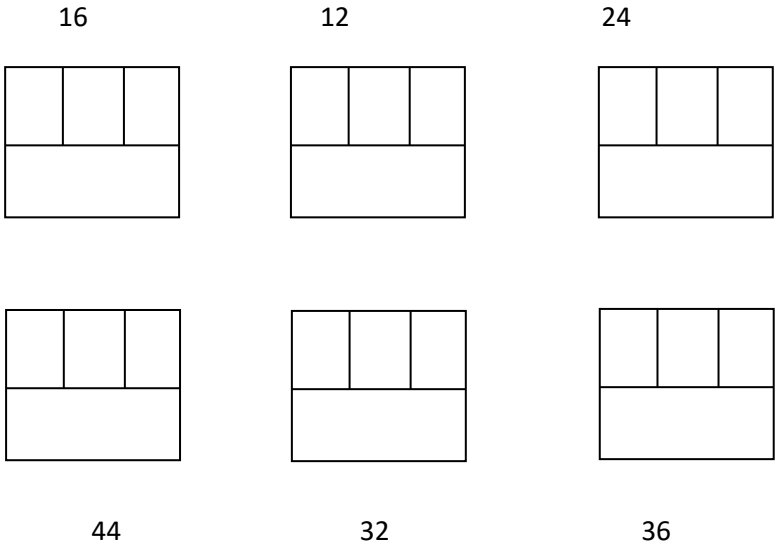
PLAQUE INDEX (After 3 months)



$$\frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$$

SCORE:

GINGIVAL INDEX by Loe & Silness 1963 (At Baseline)

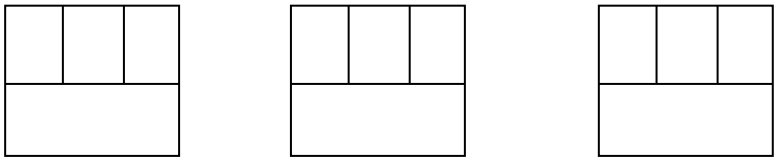


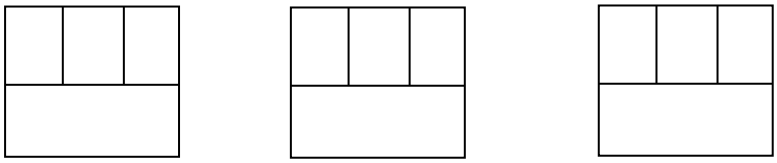
$$\frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$$

SCORE:

GINGIVAL INDEX (After 1 month)

16 12 24





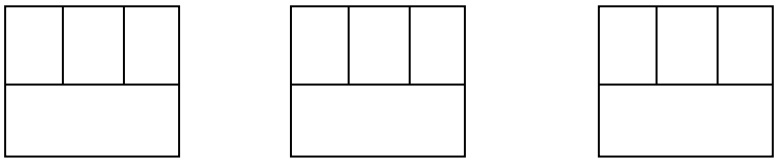
44 32 36

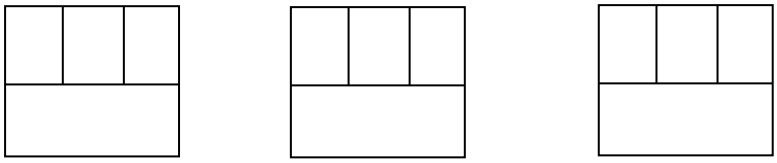
$$\frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$$

SCORE:

GINGIVAL INDEX (After 3 months)

16 12 24



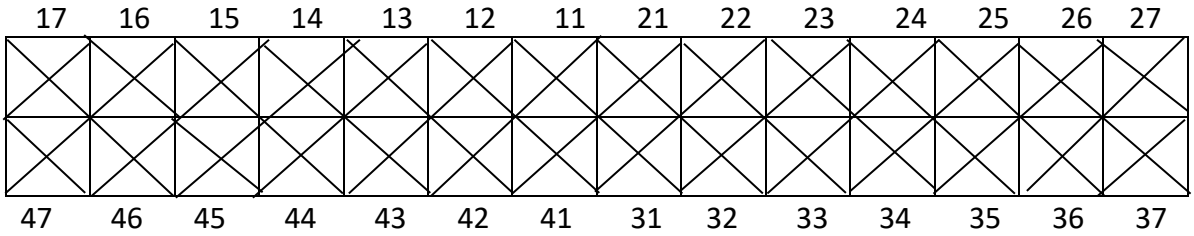


44 32 36

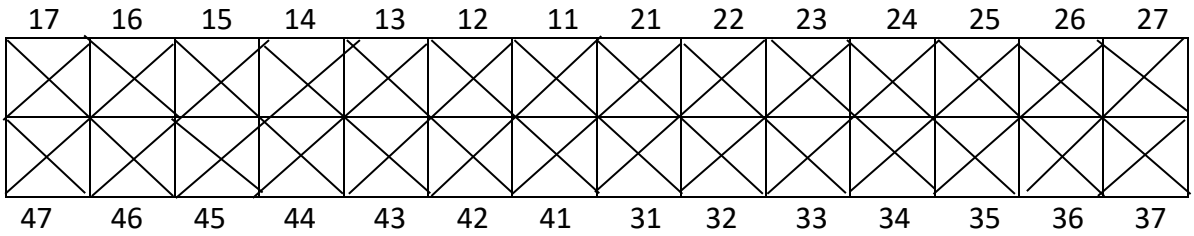
$$\frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$$

SCORE:

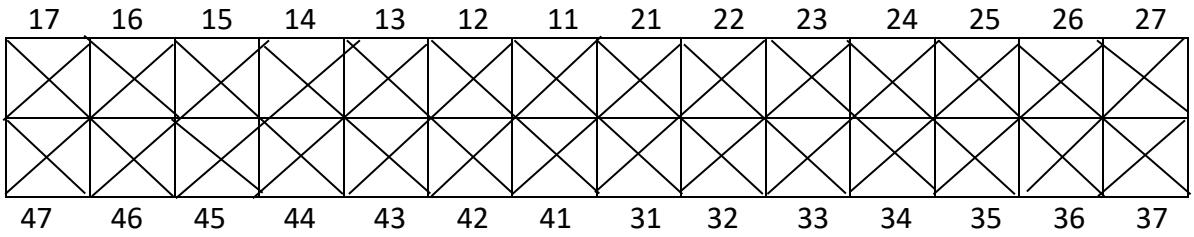
PROBING POCKET DEPTH (mm): At Baseline



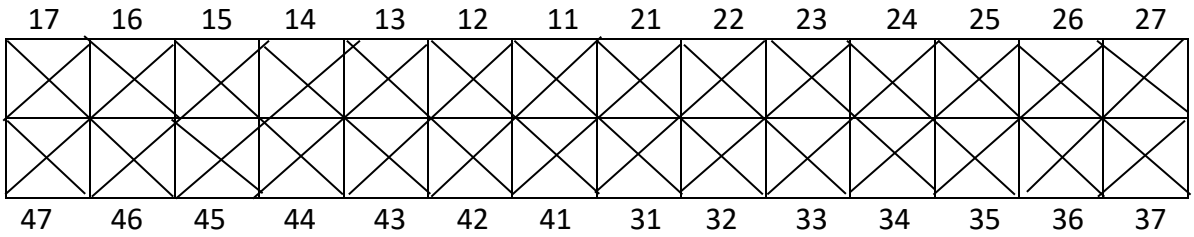
PROBING POCKET DEPTH (mm): After 1 month



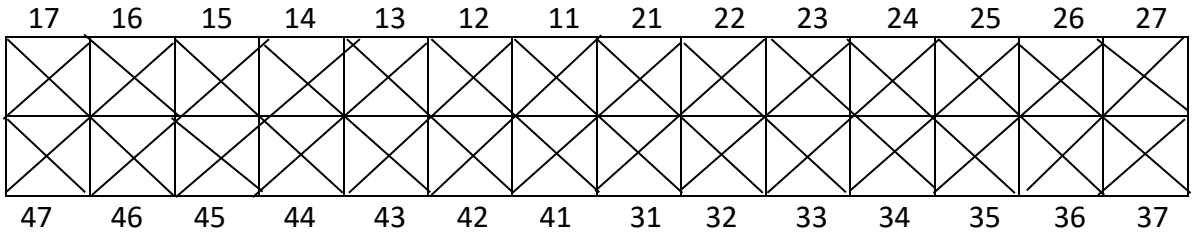
PROBING POCKET DEPTH (mm): After 3 months



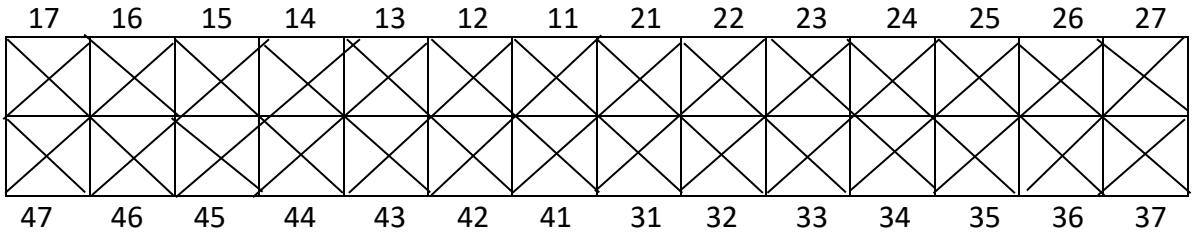
CLINICAL ATTACHMENT LEVELS (mm): At Baseline



CLINICAL ATTACHMENT LEVELS (mm): After 1 month



CLINICAL ATTACHMENT LEVELS (mm): After 3 months



Clinical Diagnosis:

Parameters	**TEST**	**CONTROL**	**TEST**	**CONTROL**
	Baseline		**After 3 months**	
GCF IL-6 Level (pg/ml)				
GCF IL-8 Level (pg/ml)				
GCF IL-10 Level (pg/ml)				

Informed Consent Form

(Confidential)

"Effect of Photodynamic Therapy as an adjunct to Non-Surgical Periodontal Therapy on Gingival Crevicular Fluid Interleukin-6, Interleukin-8 & Interleukin-10 Levels in Chronic Periodontitis: A Randomized Controlled Trial"

Mr./Master/Mrs./Miss._____ (optional)

Resident of: _____

_____aged _____ years,

Exercising my free will/choice, without any pressure/lure of incentive in any form, hereby give my consent for the project to be conducted by **Dr. _____**

I acknowledge the receipt of "patient's information sheet", and also the doctor has informed me about this research project suitably and sufficiently to my satisfaction. I agree to let my X-rays, photographs, blood investigations, other investigations to be taken as required. I agree to take part in this project and will not mix any other projects during the period of this trial. I shall report to the dental hospital or other place where called on given appointment dates and time. I shall inform the doctor on any adverse effects or unusual symptoms noticed by me. I shall co-operate with the doctors and paramedical staff, in all respects. I permit to publishing the results of my participation in this study. I shall not be given any reimbursement or compensation. I have been informed of my right to opt out of this research project at any time without giving any reason for doing so. I hereby record my consent for participation in the said trial.

(Optional information)

_____	_____	_____	_____
Patient's name	Signature/thumbprint	Date	Time
_____	_____	_____	_____
Investigator's name	Signature	Date	Time