# EFFECT OF NON SURGICAL PERIODONAL THERAPY ON GCF AND SERUM LEPTIN LEVELS IN PERIODONTALLY HEALTHY, CHRONIC PERIODONTITIS AND CHRONIC PERIODONTITIS WITH TYPE 2 DIABETES MELLITUS PATIENTS

**Dissertation submitted to**

**Maharashtra University of Health Sciences, Nashik**

**in the Partial Fulfillment of Regulations**

**for the award of the Degree of**

**MDS**

**IN**

**PERIODONTICS**

**BRANCH II**

**2019**

# CONTENTS

# LIST OF TABLES

# LIST OF GRAPHS

# LIST OF FIGURE

# LIST OF COLOUR PLATES

| SR. NO. | TITLES | PLATE NO. |
|---|---|---|
| 1 | Group I | I |
| 2 | Group II | II |
| 3 | Group III | III |
| 4 | Armamentarium for clinical examination and serum collection | IV |
| 5 | Collection of blood | |
| 6 | Collected serum sample | |
| 7 | Armamentarium for clinical examination and GCF collection | V |
| 8 | GCF Collection | |
| 9 | Deep Freezer | VI |
| 10 | Kinesis Dx Human Leptin ELISA Kit | |
| 11 | Cenrifuge Machine | |
| 12 | Vortex Mixer | |
| 13 | Microplate Washer | |
| 14 | Microplate Reader Machine | |

# LIST OF ABBREVIATIONS

| SR. NO. | SHORT FORM | FULL FORM |
|---|---|---|
| 1 | DM | Diabetes mellitus |
| 2 | AGE- RAGE | Advanced glycation end product and receptor for advanced glycation end product |
| 3 | TNF-α | Tumor necrosis factor- alpha |
| 4 | IL-6 | Interleukin 6 |
| 5 | LPS | Lipopolysaccharide |
| 6 | IRS-I | Insulin receptor substrate-I |
| 7 | FFA | Free fatty acids |
| 8 | BMR | Basal metabolic rate |
| 9 | ob | Obesity gene |
| 10 | Th1 | T helper 1 |
| 11 | (IRS) - PI3K | Insulin receptor substrate and phosphoinositide-3-kinase pathway |
| 12 | IL-2 | Interleukin 2 |
| 13 | IL-18 | Interleukin-18 |
| 14 | INF-γ | Interferon- gamma |
| 15 | TH2 | T helper 2 |
| 16 | IL-4 | Interleukin-4 |
| 17 | IL-5 | Interleukin-5 |
| 18 | IL- 10 | Interleukin-10 |
| 19 | CP | Chronic periodontitis |
| 20 | GCF | Gingival crevicular fluid |
| 21 | T2DM | Type 2 diabetes mellitus |
| 22 | PPD | Probing pocket depth |
| 23 | GR | Gingival recession |
| 24 | CAL | Clinical attachment level |
| 25 | PI | Plaque index |

| | | |
|---|---|---|
| 26 | GI | Gingival index |
| 27 | HbA1c | Glycated hemoglobin |
| 28 | SRP | Scaling and root planing |
| 29 | IR | Insulin resistance |
| 30 | SBI | Sulcus bleeding index |
| 31 | ELISA | Enzyme-linked immunosorbent assay |
| 32 | FINS | Fasting insulin |
| 33 | HOMA-IR | Homeostasis model of assessment - insulin resistance |
| 34 | HOMA- β | Homeostasis model assessment of β-cell function |
| 35 | BOP | Bleeding on probing |
| 36 | HOMA 2 | Homeostasis Model Assessment β-cell function |
| 37 | OHI-S | Simplified oral hygiene index |
| 38 | DPTT | Diabetes and Periodontal Therapy Trial |
| 39 | PISA | Periodontal Inflamed Surface Area |
| 40 | RR | Rate ratio |
| 41 | NIDDDM | Non-insulin dependent DM |
| 42 | CRP | C reactive protein |
| 43 | MetS | Metabolic syndrome |
| 44 | hs-CRP | high sensitivity C reactive protein |
| 45 | WHR | Waist-hip ratio |
| 46 | mRNA | Messenger RNA |
| 47 | AP | Aggressive periodontitis |
| 48 | HC | Healthy controls |
| 49 | IL-4 | Interleukin- 4 |
| 50 | IL-5 | Interleukin- 5 |
| 51 | IL-12 | Interleukin- 12 |
| 52 | IL-13 | Interleukin- 13 |
| 53 | IL-17A | Interleukin- 17 A |
| 54 | AMI | Acute myocardial infarction |
| 55 | GCP | Generalized chronic periodontitis |
| 56 | PD | Probing depth |
| 57 | RBS | Random blood sugar level |
| 58 | ROS | Reactive oxygen species |
| 59 | JAK | Janus kinase |
| 60 | STAT | Signal transducer and activator of transcription pathway |

| | | |
|---|---|---|
| 61 | JAK–STAT3 | Janus kinase–signal transducer and activator of transcription pathway |
| 62 | PI3K | Phisphoinositide 3Kinase |
| 63 | BBB | Blood– brain-barrier |
| 64 | SOCS-3 | Suppressor of cytokine signaling-3 |

# INTRODUCTION

The inflammatory process involving innate and adaptive immunity marks the pathogenesis of periodontitis. Infiltration of leukocytes and induced production of lytic enzymes leading to destruction of soft tissues represent this inflammatory process. Bacterial invasion and host-response both play a major role in initiation and progression of periodontal disease. Production of cytokines is the intervening mechanism that links bacterial stimulation and tissue destruction. Chemotactic cytokines i.e. chemokines stimulate recruitment of inflammatory cells as well as modulate osteoclast formation either directly or indirectly and prolong their survival. Thus, a cascade of reaction is initiated, leading the host susceptible to the pathogenic microorganisms and to other immunodeficient diseases.[1]

The interest in determining the relationship between periodontitis and systemic diseases can be accredited to the study done by Kimmo Mattila and his colleagues in 1989[2], in which they found an association between periodontitis and acute myocardial infarction. Since then, a lot of research work has been undertaken, linking periodontitis with many systemic conditions such as, diabetes mellitus (DM)[3], preterm low birth weight[4], respiratory diseases[5] and osteoporosis.[6] The association between obesity and periodontitis is well established in several investigations as obesity embodies a systemic condition capable of aggravating periodontal destruction owing to hyper inflammatory state observed in obesity which triggers the worsening of chronic diseases like periodontitis.[7]

India is known as the diabetic capital of the world. It is reported that 41 million Indians are suffering from diabetes.[8] Diabetes mellitus is a condition with a wide global prevalence and is caused by alteration of insulin dependent glucose and lipid metabolism, leading to insulin resistance and thereby lack of insulin levels. The advanced glycation end product and receptor for advanced glycation end product (AGE-RAGE) reaction occurring as a consequence of hyperglycemia is responsible for a majority of reactions leading to periodontitis such as hyper permeability of endothelial cells, hyper expression of adhesion molecules, impaired wound healing and decreased collagen production by fibroblasts. This reaction also leads to transformation of macrophages into cells with a destructive phenotype which produces pro-inflammatory cytokines such as Tumor necrosis factor- alpha (TNF-α) and Interleukin-6 (IL-6) generating the pathogenesis of periodontitis.[9]

The mechanism by which periodontitis leads to DM is attributed to the bacterial lipopolysaccharide (LPS), a potent endotoxin which enhances the production of pro-inflammatory cytokines including TNF-α which functions by adding on to insulin

resistance by phosphorylating a serine residue of insulin receptor substrate-I (IRS-I) and inhibits tyrosine phosphorylation which is necessary for insulin signal transduction. TNF-α indirectly enhances the release of other molecules which cause insulin resistance such as free fatty acids (FFA) from adipocytes. These mechanisms generate a cyclic event linking periodontitis to diabetes mellitus, establishing a two-way relationship.[10]

Likewise, obesity, an outspread concern for the modern world, is a risk factor for a number of systemic conditions like DM, periodontitis, hypertension, coronary heart disease and osteoarthritis, with a multi factorial etiology characterized by an increased basal metabolic rate (BMR).[11] Obesity leads to increase in the size of adipocytes, which undergo molecular and cellular alterations, affecting systemic metabolism by two ways: Firstly, by increasing the free fatty acid and glycerol production by adipocytes, promoting insulin resistance. Secondly, by enhancing the production of pro-inflammatory cytokines and decreasing the production of anti-inflammatory cytokines such as adiponectin which is a potent inhibitor of TNF-α induced monocyte adhesion and expression of adhesion molecules. Macrophages which are responsible for most of the cytokine production also increase in great number in obese individuals. All these mechanisms result in a low grade inflammatory state connecting obesity with diabetes mellitus and periodontitis.[12]

The adipose tissue functions not only as a reservoir of energy storage and expenditure but also regulates various complex metabolic and endocrine functions. The foremost discovery of adipocytokines such as leptin, adiponectin and resistin has taken our knowledge far beyond the function of adipocytes in regulating body weight to their function in lipid and carbohydrate mechanism as well as regulation of immune responses. Leptin, a product of obesity (ob) gene, is a cytokine like hormone and

circulates in blood as a non glycosylated peptide of 16 kDa protein.[13] It is translated as 167 amino acid protein but circulates in blood as a protein of 146 amino acid residues. As Leptin is secreted from adipose tissue, its levels correlate directly with the size of adipocytes and body fat mass. Its regulation depends on food intake and insulin secretion. Leptin has central and peripheral effects and its secretion is also modulated by sexual hormones as testosterone inhibits its secretion, while it is enhanced by ovarian sex steroids. Central actions in hypothalamus include inhibition of appetite and stimulation of energy expenditure; while peripherally it has a broad range of actions such as it increases the basal metabolic rate, influences pancreatic cell function and insulin secretion, regulates bone–marrow haematopoiesis and also affects the generation and differentiation of T helper 1 (Th1) cells in the lymph nodes.[14]

Evidence suggests that leptin is also related to insulin resistance, where increased leptin levels are found in diabetic individuals.[15] Leptin and insulin play an important role in diabetic condition by activating the insulin receptor substrate and phosphoinositide-3-kinase (IRS) - PI3K pathway, which is an essential pathway for metabolic control and their dysfunction results in impaired glucose homeostasis. Leptin is known to be pro-inflammatory, since autoimmune diseases and immune mediated disorders are associated with increased leptin levels. It's pro-inflammatory property is related to the fact that leptin has shown to modulate and increase the production of TH1 production of pro- inflammatory cytokines such as Interleukin- 2 (IL-2), Interleukin-18 (IL-18), Interferon gamma (INF-γ) and TNF-α and inhibition of T helper 2 (TH2) cytokines such as Interleukin-4 (IL-4), Interleukin-5 (IL-5) and Interleukin-10 (IL-10).[16,17]

Since leptin plays a major role in mediating inflammatory response, the relationship between leptin and chronic periodontitis has been investigated in various studies so far and a positive correlation has been found between serum leptin levels and chronic

periodontitis, where serum leptin concentration appears to be highest in patients with chronic periodontitis (CP) and lowest in healthy individuals, while a negative correlation exists between gingival crevicular fluid (GCF) leptin levels. Leptin is present in healthy gingiva and the levels decrease with the periodontal disease progression, thus indicating the possible role of leptin in developing systemic diseases.[18]

Preliminary evidence indicates a link between leptin with chronic inflammatory conditions and systemic diseases such as periodontitis and chronic migraine[19], cardiovascular diseases[20] and rheumatoid arthritis.[21]

Limited research work has been carried out in literature to evaluate the changes in serum and GCF leptin levels in periodontally healthy, CP and CP with Type 2 diabetes mellitus patients (T2DM) after non surgical periodontal therapy. Thus, the study was designed to evaluate the influence of non surgical periodontal therapy on GCF and serum leptin levels in periodontally healthy, CP and CP with T2DM patients.

# AIM AND OBJECTIVES

The present study aimed to evaluate the effects of non-surgical periodontal therapy on GCF and serum leptin levels in periodontally healthy, CP and CP with T2DM patients.

Also glued to this aim were certain objectives:

1. To evaluate the GCF and serum leptin levels in periodontally healthy patients, CP and CP with T2DM patients.
2. To evaluate and compare the levels of leptin in GCF and serum in patients with CP before and after non-surgical periodontal therapy.

3. To evaluate and compare the levels of leptin in GCF and serum in patients with CP with T2DM before and after non-surgical periodontal therapy.
4. To correlate the leptin levels in GCF and serum in patients with CP with and without T2DM before and after non-surgical periodontal therapy.

# REVIEW OF LITERATURE

Periodontal infection may have effects somewhere else in the body. Hence, it becomes important to understand the etiology and pathogenesis of periodontal diseases and its chronic, inflammatory and infectious nature. It is well known that micro organisms present in dental plaque and their metabolic products may enter the blood stream, thereby causing many systemic diseases and sometimes resulting in degenerative conditions.

Periodontal disease is a complication of DM and both are closely associated, highly prevalent chronic diseases with many similarities in pathobiology. Obesity and insulin resistance are major contributors in this regard. Obesity alters the normal metabolic and

endocrine function of adipose tissue, resulting in increased production of fatty acids, hormones, cytokines, and acute phase reactants. Elevated circulating levels of several pro-inflammatory cytokines have been found in individuals with periodontitis. Obesity and DM have been associated with an increased risk of periodontal disease. [10]

For the sake of better understanding, the review of literature has been divided into three parts-

A. Studies on interrelationship between periodontitis and DM.

B. Studies on inter-relationship between leptin, obesity and DM in association with periodontitis.

C. Studies on inter-relationship between leptin and periodontitis.

## A. STUDIES ON INTER-RELATIONSHIP BETWEEN PERIODONTITIS AND DM

**Da Cruz GA et al. (2008)[22]** assessed clinical and laboratory responses to full mouth scaling and root planing in patients with chronic periodontitis with and without Type 2 DM. 20 patients were recruited with a clinical diagnosis of generalized chronic periodontitis and were divided into two groups: Group I was experimental group which included patients diagnosed with Type 2 DM (DM group) and Group II was control group which included healthy controls without Type 2 DM (NDM group). Clinical parameters were evaluated at baseline and 3 months after therapy which included measurement of probing pocket depth (PPD), gingival recession (GR), clinical attachment level (CAL), plaque index (PI) and gingival index (GI). Blood samples were also taken at baseline and at 3 months and glycated hemoglobin (HbA1c) and fating glucose levels were determined. Baseline samples were collected and full mouth scaling and root planing (SRP) was performed and oral hygiene instructions were given to

patients. The results showed that the response to oral hygiene procedures was similar for PI and GI for both the groups. PPD significantly reduced after 3 months post therapy but no significant differences were found between the groups. Similar observations were noted for GR and CAL. The fasting glucose level showed no significant difference at baseline but significantly increased at 3 months in DM group. The HbA1c concentration did not change significantly during the study. The study concluded that clinical and laboratory responses were similar in DM and NDM groups after full mouth scaling and root planing.

**Sun WL et al. (2011)[23]** evaluated the effects of periodontal intervention on inflammatory cytokines, adiponectin, insulin resistance (IR) and metabolic control and also investigated the relationship between moderately poorly controlled glycemic control and chronic periodontitis. A total of 190 patients participated in the study. According to the patients who received periodontal treatment, all the Type 2 DM patients were divided into 2 groups; Group I: T2DM- NT group and Group II: T2DM-T group. The T2DM-NT group comprised of 75 patients (32 males and 43 females) and T2DM-T group comprised of 82 patients (35 males and 47 females). Clinical measurements at baseline and 3 months included sulcus bleeding index (SBI), PI, PPD and CAL. Blood samples were collected after overnight fasting at baseline and at 3 months and the following parameters were evaluated: serum adiponectin, TNF-α, IL-6 using enzyme-linked immunosorbent assay (ELISA) according to protocols. Blood glucose and lipid profile were tested using standard methods which included fasting plasma glucose, fasting insulin (FINS) and HbA1c. Homeostasis model of assessment - insulin resistance (HOMA-IR) and homeostasis model assessment of β-cell function (HOMA- β) were measured at baseline and after 3 months. The levels of clinical

periodontal variables improved significantly in T2DM-T group after 3 months as compared to T2DM-NT group 3 months post therapy. After 3 months, the serum levels of hsCRP, TNF-α, IL-6, FPG, HbA1c as well as TG decreased significantly in the T2DM-T group compared to those in the T2DM-NT group. FINS and HOMA-IR index decreased and adiponectin level was significantly increased in the T2DM-T group after 3 months of periodontal intervention compared to that in the T2DM-NT group, while the level of HDL-C showed a tendency to increase without a significant difference. The authors concluded that periodontal intervention can improve glycemic control, lipid profile and IR and reduce serum inflammatory cytokine levels while increase adiponectin levels in moderately poorly controlled T2DM patients.

**Rajhans NS et al. (2011)[24]** conducted a clinical study to assess the relationship between diabetes mellitus and periodontal disease. The study was undertaken in diabetic patients to find out the prevalence and severity of periodontal disease, to determine the influence of age and gender on prevalence and severity of periodontal disease, to evaluate the relationship between diabetes and periodontal disease in terms of plaque and calculus and also the duration of diabetes and glycemic status of diabetics and prevalence and severity of periodontal disease and to study the effect of glycemic status of diabetics on tooth mobility and loss of teeth. A total of 1500 patients (751 males; 749 females) between age range of 15-76 years were selected in the study who were diagnosed with diabetes mellitus and were under treatment. The patients were divided into 5 groups according to age. Group 1: 15-24 years, Group 2: 25-34 years, Group 3: 35-44 years, Group 4: 45-54 years and Group 5: 55 years and above. Oral examination included measurement of clinical parameters using Ramfjords periodontal index. Fasting and post-meal blood glucose levels were determined using auto-analyzer. Analysis of the

data showed that the prevalence of periodontal disease in diabetic patients was 86.8% and was almost equal among both the sexes. The mean periodontal index was lowest in Group 1 and highest in Group 2. There was a statistically significant correlation of plaque and gingival index with the severity of periodontal disease ($p<0.01$). The duration of diabetes mellitus and glycemic status of the patients also showed significant correlation with the .prevalence and severity of periodontal disease. 43.6% of the patients exhibited tooth mobility and this correlation was also statistically significant. 479 patients (32%) lost teeth after diagnosis of DM; the correlation being statistically significant. Thus, the authors concluded that poorer the glycemic status and longer the duration of diabetes, the greater is the prevalence and severity of periodontal disease.

**Modi CS et al. (2012)[25]** compared the clinical and metabolic changes in terms of HbA1c in response to periodontal treatment in T2DM patients at baseline and 3 months. 50 patients with age range between 35-70 years, diagnosed with Type 2 DM were recruited in this prospective, interventional, comparative, clinical study. The patients were equally divided into two groups as: The Treatment (test group) and The Non-Treatment (control group). The study group underwent complete clinical periodontal examination and clinical parameters recorded were PPD, PI, GI and SBI. Blood investigations were carried out which included HbA1c test. The probing pocket depth decreased significantly in the test group after 3 months while it increased significantly in the control group. The mean PI score significantly declined in both the groups at 3 months. Similar trend was followed by GI and SBI. The mean HbA1c values reduced after 3 months of periodontal therapy in the test group and the results were statistically significant. The reduction in HbA1c was not statistically significant in the control group. The authors concluded that clinical and metabolic improvements following non

surgical periodontal therapy depict a strong indicator of correlation between periodontitis and T2DM.

**Monea A et al. (2012)[26]** investigated the histologic changes that occur in the periodontium of patients with T2DM without signs of periodontal disease and to establish the influence of this systemic condition upon periodontal structures. The study population consisted of 2 groups of both the sexes- The experimental group: 12 diabetic patients, with age range of 35-58 years with no signs of established periodontal disease. The control group: 10 healthy patients, with age range of 32-50 years. Harvested tissue samples consisted of soft tissues (gingival fibromucosa) and were fixed in Lillie neutral formalin for maximum 5 days. The microscopic aspect of the experimental group presented an epithelium of variable thickness, occasionally thin, with rare sites of ulceration and acanthosis. The surrounding connective tissue presented ecstatic blood vessels surrounded by rich inflammatory infiltrate, mainly lymphoplasmocytic. Also, a pronounced mitotic activity was observed in the basal layer of the epithelium accompanying the inflammatory infiltrate. The capillaries and venules were dilated. Whereas biopsies of control group showed a squamous epithelium of normal histologic aspect. The underlying connective tissue formed small papillae that enter through epithelium (dermal papillae). Thus it was concluded from the observations that diabetic patients presented distortion in periodontal attachment, with changes in both epithelium and connective tissue, when compared to healthy controls, suggesting that diabetes mellitus has an independent effect on periodontal tissues.

**Engebretson SP et al. (2013)[27]** conducted a randomize controlled clinical trial to determine if periodontal treatment reduces HbA1c levels in patients with T2DM and

moderate to advanced CP. 514 participants were enrolled and equally divided into treatment group (n=257) and control group (n=257) in this 6 month, single masked, randomized, multi-center clinical trial. The treatment group received scaling and root planing as well as supportive periodontal therapy at 3 and 6 months whereas control group did not receive any treatment for 6 months. PPD, CAL, bleeding on probing (BOP), GI, HbA1c, fasting glucose and the Homeostasis Model Assessment β-cell function (HOMA 2) were evaluated at baseline and 6 months. The results showed that the HbA1c values did not change significantly in both the groups. Using linear regression models, all clinical parameters improved at 3 months and were sustained at 6 months in the treatment group but not in the control group. The mean PPD improved in the treatment group compared to control group. BOP decreased significantly in both the groups. Similar results were obtained for CAL and GI. The changes in fasting glucose and HOMA 2 β-cell function (% β) remained stable with no statistically significant changes in both the groups after 6 months. It was concluded from the study that non-surgical periodontal therapy did not improve glycemic control in patients with diabetes mellitus and moderate to advanced periodontitis.

**Perayil J et al. (2014)[28]** compared the HbA1c levels in non-diabetic healthy patients and patients with periodontitis, before and after non-surgical periodontal therapy. This comparative study was done on 60 non-diabetic patients within age range of >35 to <65 years. Patients were equally divided into 2 groups; Group A: 30 patients without periodontitis and Group B: 30 patients with periodontitis. HbA1c levels, BMI and clinical parameters including OHI-S, GI, PPD and CAL were recorded at baseline and 3 months. All patients received SRP. At baseline Group B had significantly higher values of simplified oral hygiene index (OHI-S), GI and PPD than Group A. Statistically

significant difference was found in HbA1c levels at baseline in Group B as compared to Group A. When comparing post-intervention parameters in both the groups, it was found that at the end of 3 months, no statistically significant difference was found in OHI-S, GI, PPD and HbA1c in Group A. Group A had no clinical attachment loss at the end of 3 months, whereas in Group B statistically significant reductions were found in OHI-S, GI, PPD, CAL and HbA1c. Thus it was concluded that HbA1c levels of non-diabetic patients with periodontitis was significantly reduced 3 months after non-surgical periodontal therapy, although they did not reach the same level as that of non-diabetic patients without periodontitis.

**Michalowicz BS et al. (2014)[29]** explored the associations between baseline characteristics and periodontal treatment response in patients with type 2 DM who participated in Diabetes and Periodontal Therapy Trial (DPTT). The study focused on patient related factors rather than tooth related factors to assess a person's likelihood of responding to treatment. This multi centered, 6 month, single-masked, randomized controlled trial was designed to test 473 participants which were divided into 2 groups; treatment group: 240 participants and control group: 233 participants. The primary outcome of this trial was to assess change in HbA1c levels after periodontal intervention. Results of multivariate analyses showed that participants in the treatment group, those with higher mean baseline PD and BMI experienced greater mean PD reductions than participants in the control group. When controlled for all other factors in the model, participants who were obese experienced 0.10 mm greater reductions in PD than their counterparts who were not obese. As with PD change, participants who were obese, experienced greater reduction in BOP than non-obese participants. Pearson product moment correlations between change in PD, CAL, BOP and HbA1c status

indicated weak correlations which were statistically insignificant. Similar weak and statistically insignificant correlation was found between changes in clinical parameters and BMI. Thus this study demonstrated that baseline disease severity is associated with the magnitude of clinical response after non surgical periodontal therapy in people with T2DM.

**Kaur PK et al. (2015)**[30] assessed the effect of non-surgical periodontal therapy in patients with T2DM and CP as well as differences in clinical periodontal and metabolic responses to periodontal therapy in patients with good glycemic control, poor glycemic control and non- diabetic individuals. This randomized controlled clinical trial was conducted in a total of 100 patients, who were classified as : 1) 23 individuals with good glycemic control who received SRP (TG Group); 2) 27 individuals with poor glycemic control who received SRP (TP Group); 3) 25 individuals with good glycemic control who did not received SRP (NTG Group) and 4) 25 individuals with poor glycemic control who did not received SRP (NTP Group); 5) 25 non-diabetic individuals who received SRP ( No DM group). Participants were assessed at baseline, 3 months and 6 months. Plasma lipid profile, glucose level, HbA1c levels, liver enzyme levels and thyroid functions were evaluated. Anthropometric measurements were also taken and clinical measurements included measurement of PPD, CAL, BOP and PISA (Periodontal Inflamed Surface Area). Improvements in periodontal parameters were significantly greater in T group than NT group. SRP resulted in statistically highly significant improvements in No DM, TG and TP group. The NTG and NTP group, which did not receive periodontal treatment showed deterioration of periodontal status over a period of 6 months. Improvements in HbA1c levels were significantly higher in T group than in NT group. Stratification of treatment group on the basis of HbA1c

revealed that decrease in HbA1c was significant for patients with poor glycemic control (TP group), while a statistically significant increase was observed in NT group. Multivariate linear regression showed that being in a treatment group and baseline HbA1c level were independently and significantly associated with improvement in glycemic control over 6 months. The authors concluded that non surgical periodontal therapy improved glycemic control and periodontal health in patients with T2DM. However, patients with poor glycemic control had less clinical improvement than those without diabetes and those with good glycemic control.

**Quintero AJ et al. (2018)[31]** evaluated the impact of two non-surgical periodontal treatment modalities on metabolic and clinical periodontal parameters in patients with T2DM, poor glycemic control and chronic periodontitis. This randomized controlled clinical trial consisted of 93 patients with T2DM with HbA1c >7%, having chronic periodontitis. One group received SRP in multiple sessions, quadrant by quadrant (Q by Q) and the second group in one stage i.e. within 24 hours. HbA1c decreased by 0.48% in the Q by Q group and by 0.18% in the one-stage group at 6 months. After therapy, patients with an initial HbA1c < 9% showed an increase of 0.31% , compared with a decrease of 0.88% in those with an initial HbA1c ≥ 9%. Periodontal parameters improved significantly ($p < 0.0001$) post-therapy, with similar results for both treatment modalities. Hence, the results suggested that periodontal therapy had the greatest impact on HbA1c reduction on patients with an HbA1c > 9% regardless of treatment modality.

**Myllymaki V et al. (2018)[32]** studied the association between periodontal condition and development of T2DM from a 15- year follow-up study. 395 patients were recruited in this population based follow-up study. The adjusted rate ratios (RR) with 95%

confidence intervals for the incidence of T2DM among patients with 4-5 mm deep periodontal pockets, among patients with 6 mm deep or deeper periodontal pockets, and among edentulous patients were 1.32, 1.56 and 1.00 respectively, compared to periodontally healthy patients. Statistically significant association between periodontal condition (the number of sites with deepened [4 mm deep or deeper] periodontal pockets and incident T2DM was found among dentate patients. The adjusted RR per site with deepened periodontal pocket was 1.02. Thus, the authors concluded that poor periodontal condition may be a predictor of the development of T2DM.

## B. STUDIES ON INTER-RELATIONSHIP BETWEEN LEPTIN, OBESITY AND DM IN ASSOCIATION WITH PERIODONTITIS

**Haffner SM et al. (1996)[33]** conducted a study to explore whether patients with Non-insulin dependent DM (NIDDDM) have an altered level of serum leptin concentration. For this, the study population was divided into 3 groups; 1) 50 Mexican- Americans with NIDDDM; 2) 50 non- diabetic Mexican- Americans matched with age and sex (control group 1); 3) 50 non- diabetic Mexican- Americans matched with age and sex and BMI to the diabetic Mexican- Americans (control group 2). Radioimmunoassay was used to determine leptin levels. The diabetic patients had slightly higher leptin concentrations than the non- diabetic controls (control group 1) but the results were not statistically significant. The leptin levels in diabetic patients were identical with the non- diabetic control group 2 which was matched with BMI. When the groups were compared by gender, women had significantly higher leptin levels than men. The correlation between BMI and leptin concentration was also similar in each group.

Multiple linear regression model with plasma leptin concentration as dependent variable showed that increased BMI, female sex and age were associated with higher plasma leptin concentration. Hence, it was concluded that leptin concentrations were not different in diabetic and non- diabetic patients and the association of leptin with obesity was similar in diabetic and non- diabetic patients.

**Kardesler L et al. (2010)[34]** performed a study to evaluate the effects of initial periodontal therapy on clinical periodontal parameters, glycemic control, and systemic inflammatory mediator levels in patients with Type 2 DM and CP. 13 well controlled (HbA1c <7%) and 12 poorly controlled (HbA1c >7%) patients with DM and CP and 15 systemically healthy patients with CP participated in the study. Blood samples were collected at baseline and after 1 and 3 months of periodontal treatment from all the patients. All the study groups showed similar improvements in clinical parameter. The HbA1c levels decreased significantly in poorly controlled diabetic group, whereas the changes were not significant in well controlled diabetic group. CRP and TNF-α levels showed insignificant reduction in all the groups. Leptin and adiponectin levels were similar at all time points in the study , however leptin levels decreased at 3 months and adiponectin levels increased significantly at 3 months in the systemically healthy group. The authors thus concluded that decrease in serum leptin levels and increase in serum adiponectin levels after periodontal therapy may be a function of glycemic control in patients with type 2 DM.

**Bandaru P et al. (2011)[35]** conducted a study to examine the association between plasma leptin levels and diabetes mellitus in a large nationally representative sample of

US adults after adjusting or major confounders. 5,599 individuals participated in the study (54.7% women), 395 of whom had diabetes mellitus. Fasting blood samples were collected from all the participants and BMI was measured. Higher plasma leptin levels were initially found to be associated with diabetes mellitus. However, when additionally adjusted for BMI, the association between plasma leptin levels and diabetes mellitus disappeared in both men and women. No association was found between increasing plasma leptin levels and diabetes in normal weight individuals. Plasma leptin levels were significantly and positively related to C reactive protein (CRP) levels. Thus, higher plasma leptin levels were not independently associated with diabetes mellitus after adjusting for BMI.

**Mohammadzadeh G et al. (2012)[36]** evaluated the variations in serum leptin levels in non-obese with T2DM and association between these levels and anthropometric and clinical characteristics of T2DM in comparison with the healthy control group of non-diabetic patients. The diabetic group which comprised of 41 middle aged non-obese individuals with T2DM (21 women and 20 men) were enrolled in the study. The non-diabetic group comprised of 39 middle aged non-obese individuals (21 women and 18 men) with fasting blood glucose concentration <110 mg/dl and HbA1c < 5.5%. Fasting lipid profile, serum leptin, HbA1c, insulin and glucose levels were measured by standard methods. Serum leptin levels were found to be lower in diabetic patients in men as compared to women, while it was significantly and positively correlated with BMI ($p<0.0001$) and also with insulin and HOMA-β in both groups. Furthermore, leptin related to HOMA-IR was observed only in diabetic group. Leptin showed a negative correlation with waist-hip ratio and non- diabetic patients. It was speculated from the

results that serum leptin levels in diabetic patients may be consequence of male gender; moreover serum leptin levels are influenced differently in women than in men.

**Altay U et al. (2013)**[37] conducted a study with an aim to evaluate short-term changes in systemic inflammatory, lipid and glucose parameters in obese patients after periodontal treatment. The study population comprised of 22 dyslipemic patients with obesity and 24 healthy individuals without obesity with generalized chronic periodontitis. The clinical and biochemical measurements were recorded at baseline and after 3 months of non-surgical periodontal treatment. There were significant improvements in clinical parameters at 3 months in both the groups. Significant reduction in TNF-α, IL-6 and leptin levels were observed in group with obesity, whereas only IL-6 levels showed significant reduction in non-obese group. Conversely, CRP levels did not show significant changes. No significant change in serum lipid profile was noted in both the groups. Serum fasting blood glucose and insulin decreased in the obese group, however, the values did not reach the level of clinical significance. Thus, the authors concluded that non-surgical periodontal treatment causes decrease in levels of some pro-inflammatory cytokines and may be associated with a decrease in insulin resistance in obese population.

**Zimmermann GS et al. (2013)**[38] investigated the local and circulating levels of adipocytokines in obese and normal weight individuals with CP. Based on the periodontal and anthropometric parameters, 78 participants were divided into following groups; NWNP group: Normal weight, non-periodontitis group (n=20); NWCP group: Normal weight, chronic periodontitis group (n=20); ONP group: Obese, non-

periodontitis group (n=18) and OCP group: Obese, chronic periodontitis group (n=20). The levels of circulating adipocytokines were evaluated in GCF and serum using ELISA kits. The serum resistin levels were higher whereas adiponectin levels were lower in periodontitis than in NP group. The NWNP group exhibited lowest serum leptin concentration. The ONP and OCP groups exhibited higher TNF-α levels than NWNP and NWCP groups, whereas the levels in GCF were higher in OCP group than in NWCP group. Serum levels of IL-6 and leptin were correlated with the OCP group, with odds ratios of 0.99. Individuals in the ONP group presented the highest concentration of leptin in GCF. Hence, it was concluded that periodontitis mainly influenced the circulating levels of resistin and adiponectin, whereas both obesity and periodontitis affected the circulating levels of leptin in favor of pro inflammation.

**Gonçalves TE et al. (2014)[39]** investigated the effects of SRP on clinical parameters and circulating levels of leptin and adiponectin in obese patients with chronic periodontitis. 24 obese and 24 non-obese patients with CP were submitted to SRP. Clinical and biochemical parameters were assessed at baseline, 3 and 6 months. The results showed that SRP improved the clinical parameters at 6 months in both the groups. However, the non-obese patients showed a greater reduction in probing depth at 6 month post therapy. Leptin levels were higher in obese than non- obese groups at all time points, while adiponectin levels showed no difference between the groups. No changes in serum leptin and adiponectin levels were noted in both the groups post- therapy. Thus, the authors concluded that obese patients with CP presented lower reduction in PD than non-obese patients at 6 months post SRP. Furthermore, the treatment did not affect the circulating levels of leptin and adiponectin in any group.

**Jing Ling X et al. (2014)[40]** explored the difference in serum ratio of leptin/adiponectin among patients with diabetes and periodontitis, patients with periodontitis only and healthy patients and to find out the ratio between this ratio and periodontal parameters. A total of 45 patients were enrolled and were equally divided into 15 patients with diabetes and periodontitis, 15 chronic periodontitis patients and 15 healthy patients. The patients in T2DM+ CP group showed higher plasma fasting glucose levels than the other two groups. Leptin levels and the ratio of leptin/adiponectin were found to be highest in T2DM+ CP group whereas they were lowest in healthy group. Inversely, adiponectin levels were highest in healthy group and lowest in T2DM+ CP group. The ratio of leptin/adiponectin showed a positive correlation with periodontal parameters. Thus, the study concluded that chronic periodontitis could influence the level of adipokines in serum and change the ratio of leptin/adiponectin, and the effect would be enhanced combining with T2DM.

**Ghallab NA et al. (2015)[41]** investigated the protein and gene expression of leptin and visfatin in gingival tissue from patients with CP, T2DM with CP and healthy individuals. 50 individuals were divided as: 10 healthy individuals, 20 patients with CP and 20 patients with CP and T2DM. Leptin and visfatin protein expression in gingival tissues was determined using ELISA, and messenger RNA (mRNA) expression was measured via real-time polymerase chain reaction. The highest mean leptin mRNA and protein expression was observed in control group and was significantly different from the other two groups. Gingival tissues from patients with CP and T2DM had a significant increase in visfatin and a decrease in leptin gene and protein expression compared with both controls and patients with CP. Thus, the expression of leptin and

visfatin in gingival tissues suggest a possible role of these adipokines in the pathogenesis of CP and T2DM.

**Mendoza-Azpur G et al. (2015)[42]** assessed the serum levels of leptin, adiponectin and TNF-α in obese and normal weight patients with and without CP. 93 patients were enrolled and divided into the following groups: 30 periodontally healthy NW patients; 18 NW patients with CP, 21 periodontally healthy obese patients and 24 obese patients with CP. Obese patients with CP showed significantly more bleeding sites than the other 3 groups. The periodontally healthy obese patients showed similar levels of adiponectin and leptin than obese patients with CP, but these levels were significantly higher than NW patients with and without CP. For TNF-α, no statistically significant differences were found between the groups. MLR analyses showed that obesity was positively associated with the percentage of sites with bleeding on probing, with an odds ratio of 0.93. The authors thus concluded that the serum leptin and adiponectin and TNF-α were not influenced by CP. Obese patients showed almost 10% more sites with BOP. In CP group, obese patients presented significant more BOP than normal weight individuals.

**Goncalves TE et al. (2015)[43]** evaluated the effects of SRP on GCF and serum levels of adipokines in patients with chronic periodontitis with and without obesity. 20 patients with obesity and 20 patients without obesity, all with chronic periodontitis received SRP. The GCF and serum levels of leptin, adiponectin, TNF-α and IL-6 were evaluated at baseline, 3, 6 and 12 months post therapy. SRP reduced TNF-α levels in deep sites and increased the concentration of adiponectin in shallow sites of non-obese patients.

The concentrations of leptin and TNF-α were higher in obese patient post SRP. No statistically significant differences were found in adipokines levels after SRP. Patients with obesity exhibited higher leptin levels at all time points and IL-6 at 3 months post therapy. Thus, it was concluded that SRP did not affect the circulating levels of adipokines in patients with or without obesity.

**Chen HD et al. (2017)[44]** investigated the relationship between fasting serum leptin levels and metabolic syndrome (MetS) in patients with T2DM. This prospective cross-sectional study included 140 patients with T2DM, amongst which 95 patients had MetS and 66 patients had obesity. Anthropometric and biochemical measurements were done. Results showed that female gender, hypertension, BMI, triglyceride levels, high sensitivity C reactive protein (hs-CRP), HbA1c, insulin level, HOMA-IR and leptin levels were higher, while HDL levels were lower in patients with T2DM who had MetS. Higher serum leptin levels were significantly correlated with BMI levels in our DM patients. Thus, the study showed that the leptin levels were positively associated with MetS in patients with T2DM. In addition, body fat mass, log-hs-CRP, and log-insulin are positively correlated with leptin levels.

**Diwan AG et al. (2018)[45]** aimed to analyze the serum levels of leptin and adiponectin in participants with T2DM and obesity and their correlation with hypertension and dyslipidemia. 50 patients with diabetes mellitus and 50 controls were enrolled in the study. Serum leptin and adiponectin levels were found to be significantly higher in diabetics as compared to non diabetic patients. Female gender showed comparatively higher leptin levels as compared to males but this difference was statistically

insignificant with respect to adiponectin levels. It was also found that low serum adiponectin was associated with a higher incidence of hypertension while high serum leptin was associated with higher incidence of hypertension. Thus, the authors concluded that low serum adiponectin and high serum leptin levels are associated with increased risk of T2DM, obesity, increased waist-hip ratio (WHR), and hypertension.

## C. STUDIES ON INTER-RELATIONSHIP BETWEEN LEPTIN AND PERIODONTIIS

**Karthikeyan BV et al. (2007)[46]** conducted a study with a two- fold aim to assess the concentration of human leptin in GCF and serum and to find out their association, if any with periodontal health and disease. The study group comprised of 42 patients (30-39 years) who fell in the normal BMI chart of WHO and were divided into 3 groups; Group I: 14 patients with clinically healthy periodontium; Group II: 14 patients exhibiting chronic gingivitis with no attachment loss and Group III: 14 patients exhibiting chronic periodontitis with attachment loss. GCF and serum samples were collected and the samples were assessed for leptin levels sing commercially available ELISA kit. Spearman's rank correlation between the clinical parameters and GCF and serum leptin levels showed significant negative correlation between GCF leptin levels and a significant positive correlation between serum leptin levels and clinical parameters. Kruskal–Wallis test was done to compare the mean leptin concentration in GCF and serum at different CAL levels and the results showed a significant reduction in GCF and significant increase in serum leptin levels as CAL progressed. Thus, the results

suggested that greater the periodontal destruction, lesser is the GCF leptin concentration and greater the serum leptin concentration.

**Shimada Y et al. (2010)**[47] conducted a study which aimed at investing role, periodontal disease might play in production of serum leptin by determining the role of periodontal treatment on serum leptin, IL-6 and CRP. A total of 33 patients with chronic periodontitis and 18 healthy controls were recruited in the study. All CP patients received 2-3 visits of scaling and root planing within a period of 2 months. Analysis of data using Mann-Whitney U test revealed significant differences in serum leptin, IL-6 and CRP in healthy and CP patients. The serum leptin level was associated with mean probing depth, CAL, alveolar bone loss and BMI. Also, significant association was found between serum leptin and IL-6 as well as CRP levels. After non-surgical periodontal treatment, serum leptin, IL-6, and CRP levels were significantly decreased. Thus, periodontal treatment is effective in reducing serum leptin, IL-6, and CRP levels, suggesting that serum leptin, IL-6, and CRP levels could be mediating factors that connect metabolic syndrome and periodontitis.

**Vadvadgi VH et al. (2012)**[48] assessed the concentration of human leptin in GCF and serum within healthy and diseased gingiva to explore the possibility of using leptin levels in GCF and saliva as a biochemical marker of periodontal disease progression. 90 patients (30-39 years) were selected and were equally divided into 3 groups of 30 patients (15 males and 15 females) each. Group I: Healthy periodontium; Group II: Gingivitis and Group III: Periodontitis. GCF and serum samples were collected to determine the leptin concentrations. The highest mean GCF leptin concentration was

found in Group I and least in Group III. In contrast, the highest mean serum leptin concentration was found in Group III, while least serum leptin concentration was found in Group I. Further, when leptin levels were in GCF and serum were tested or correlation with clinical parameters i.e. modified gingival index, periodontal disease index and CAL, a negative correlation or GCF leptin and a positive correlation for serum leptin concentration was observed. Thus, the results indicate a statistically significant decrease in GCF leptin concentration and increase in serum leptin concentration as the periodontal disease activity progressed.

**Ay ZY et al. (2013)[49]** investigated the relationship of serum leptin concentration and their proportional relationship with pro and anti- inflammatory mediators in patients with Aggressive Periodontitis (AP). The study population comprised of a total of 12 patients; 6 patients with AP and 6 Healthy Controls (HC). Periodontal parameters were measured and blood samples were collected for analyses of cytokines and leptin levels sing ELISA. Results of the study showed that all the clinical parameters were significantly higher in AP group. The serum leptin levels, though higher in AP group, did not show statistically significant difference between the groups. In the AP group, the serum transforming growth factor- beta (TGF-β1) and IL-2 concentrations were significantly higher and the Interleukin- 4 (IL-4), Interleukin- 5 (IL-5), Interleukin- 12 (IL-12), Interleukin- 13 (IL-13), and Interleukin- 17 A (IL-17A) concentrations were significantly lower than in the healthy control group. Mann–Whitney U test revealed that the ratios of leptin to IL-12, IL-13, and IL-17A were lower and that to TGF-β1 was higher in the AP than the HC group. Within the limitation of small sample size, the study determined a proportional relationship between leptin concentration and pro and anti- inflammatory mediators in AP patients.

**Gundala R et al. (2014)[50]** conducted a study with an attempt to assess the concentration of serum leptin associated with or without Acute Myocardial Infarction (AMI). 120 patients participated in this cross- sectional study within the age range of 30-60 years. The patients were equally divided into 4 groups (30 each). Group I: Healthy controls; Group II: patients diagnosed with AMI only; Group III: patients with Generalized Chronic Periodontitis (GCP) only and Group IV: patients diagnosed with AMI plus GCP. Clinical periodontal parameters, BMI were recorded and blood samples were drawn for leptin analyses. The results revealed a strong correlation of serum leptin concentration to BMI in AMI, GCP an AMI+GCP groups. Serum leptin levels were significantly elevated in AMI, GCP an AMI+GCP groups compared to healthy controls ($p<0.05$). Significant association between serum leptin concentration and clinical periodontal parameters was also observed. Also, the periodontal parameters were also elevated significantly in AMI+GCP and GCP groups. Thus, the authors concluded that elevated serum leptin concentration was associated with increased BMI and GCP and AMI may serve as risk marker for these conditions.

**Meharwade VV et al. (2014)[51]** conducted a split-mouth study to evaluate the effects of SRP with and without local drug delivery system on GCF leptin levels in chronic periodontitis patients. 90 sites from total 30 non-obese participants were selected and divided as; Group I: 30 healthy sites receiving no treatment; Group II: 30 sites with periodontitis receiving SRP; Group III: 30 sites with periodontitis receiving SRP with tetracycline local drug delivery. GCF samples were collected at baseline and at 15 and 45 day recall visits (D0, D15, and D45). The reductions in clinical periodontal parameters at different time intervals were statistically significant in Groups II and III at D 45 whereas the difference between D15 and D45 were not statistically significant.

The variation in the mean GCF leptin level at D0, D15, and D45 among the study groups was statistically not significant. However the variation in GCF leptin concentration was not significant in Group II, it was statistically significant in Group III. The reduction in the mean GCF leptin level was statistically significant between D0 and D15 followed by a significant increase between D15 and D45 for Group III. Thus, there was progressive improvement in clinical parameters after periodontal therapy with a more significant reduction in leptin in Group III than in Group II sites, following which there was re-elevation of GCF leptin to almost the pretreatment level, which suggested that leptin not only mediated periodontal local inflammation but also had systemic effects.

**Al-Azawy VS et al. (2015)**[52] performed a study to evaluate the serum levels of leptin and adiponectin in CP patients, to determine their association with clinical periodontal parameters and assess the correlation between leptin and adiponectin. 3 patients with CP and 25 healthy patients were enrolled in the study. Clinical periodontal parameters recorded were PI, GI, PPD, CAL and BOP and leptin and adiponectin concentrations were analyzed using ELISA. The data analyzed revealed a significant elevation in mean leptin concentration in patients with chronic periodontitis than with their healthy counterparts and statistically significant decrease in serum adiponectin concentration. The ratio of leptin: adiponectin was significantly higher in chronic periodontitis patients. Additionally, these findings did not observe any significant correlation between serum leptin, adiponectin, and ratio of leptin/adiponectin with clinical periodontal parameters. Thus, the study demonstrated that serum levels of leptin and adiponectin play a crucial role in pathogenesis of periodontitis and the relative leptin/ adiponectin ratio appears to be indicative of disease occurrence.

**Selvarajan S et al. (2015)[53]** conducted a study with the objective to evaluate and compare the GCF concentrations of leptin in periodontally healthy and diseased patients; to compare GCF leptin levels with respect to BMI and to obtain an insight into leptin's possible role in initiation and progression of periodontal disease. 60 patients within age range of 30-50 years were selected and divided into 3 groups of 20 patients each. Group I with clinically healthy periodontium; Group II with clinical signs of gingivitis and Group III with clinical signs of periodontitis. GCF samples were collected extra-crevicularly using black color-coded 1-5 µl calibrated volumetric micro capillary pipettes, and samples were analyzed for leptin using a commercially available ELISA kit. The concentration of GCF leptin was significantly higher in Group I than those in Group II and Group III. Pearson Correlation Coefficient test to compare GCF leptin concentration with clinical parameters revealed that in all groups, leptin and clinical parameters were negatively correlated and not statistically significant. No statistically significant difference was found when comparing the mean values of leptin between BMI groups in each study group. Thus, the authors concluded that as there was increase in periodontal disease progression, there was a substantial decrease in GCF leptin concentration, suggesting a protective role of leptin with regard to periodontal health.

**Purwar P et al. (2015)[54]** conducted a comparative quantitative cross-sectional study to assess the concentration of leptin in saliva and serum in periodontally diseased and healthy sites and to find their association, if any with periodontal health and disease. 84 patients participated in the study; 44 patients with generalized CP and 40 healthy controls. Patients with CP demonstrated significantly higher BOP, PI, GI and CAL. Salivary leptin levels were significantly lower in CP patients than healthy controls. Inverse correlation was observed for serum leptin levels. A significant negative

correlation for salivary leptin and a positive correlation for serum leptin were found with PD. The results thus suggested that leptin concentrations in saliva and serum are significantly altered in CP; and relate closely to the current disease activity.

**Khorsand A et al. (2015)**[55] compared the salivary leptin levels in patients with advanced periodontitis and healthy patients. 16 patients with advanced periodontitis (26-37 years) and 16 healthy patients were enrolled in this cross-sectional study. Statistical analysis revealed statistically significant higher values of PI, PPD, CAL and BOP in patients with advanced periodontitis than healthy patients. The mean salivary leptin levels were higher in patients with advanced periodontitis. The results of two-way ANOVA revealed no statistically significant difference between males and females. The effect of advanced periodontitis on salivary leptin levels was found to be highly significant. Hence, it can be concluded that assessment of salivary leptin levels can be done as non-invasive and simple method to determine the susceptibility of patients to advanced periodontitis.

# MATERIALS AND METHODS

The present interventional study was carried out to evaluate the effects of non-surgical periodontal therapy on GCF and serum leptin levels at baseline and after 6 months in periodontally healthy, chronic periodontitis and chronic periodontitis with type 2 diabetes mellitus patients.

Ninety patients within the age range of 30- 50 years visiting the Department of Periodontology, of our Institute were recruited in this study. The study design was reviewed and approved by the Institutional Ethics Committee and is in accordance with the Helsinki Declaration. Prior to the initiation of the study an informed consent was obtained from those who agreed to participate voluntarily.

## Study groups

A dental and medical history was recorded for the selected patients and an intraoral examination was conducted by a single examiner. Patients were then categorized into 3 groups of 30 patients in each on the basis of periodontal parameters including Probing pocket depth (PPD), Clinical attachment level (CAL), Plaque index (PI) [Silness and Loe 1964], Gingival index (GI) [Loe and Silness, 1963], radiographic evidence of bone loss, HbA1C levels and RBS levels.

**1. Group I (Systemically and periodontally healthy patients)**

Patients with clinically healthy periodontium, GI = 0, PD≤3mm and CAL = 0 with no radiographic evidence of bone loss.

HbA1c levels< 6.5%, RBS levels <160 mg/dl.

**2. Group II (Chronic periodontitis patients without DM)**

Generalized moderate to severe chronic periodontitis patients with GI > 1, >30% of sites having PPD of ≥5mm, positive BOP and CAL of ≥5mm with radiographic evidence of bone loss.

HbA1c levels < 6.5 % and RBS levels < 160 mg/dl.

**3. Group III (Chronic periodontitis patients with DM)**

Generalized moderate to severe chronic periodontitis with Type 2 DM patients with GI score > 1, >30% of sites having PPD of ≥ 5mm, positive BOP & CAL of ≥5mm with radiographic evidence of bone loss.

HbA1c levels between 6.5- 8 % and RBS levels ≥ 160 mg/dl.

All the patients were evaluated for the detection of leptin levels in GCF and Serum, HbA1c and RBS levels at baseline and 6 months as well status of the clinical parameters at baseline, 3 and 6 months after Phase I periodontal therapy. **(Color Plates I, II and III)**

# Patient selection criteria

## Inclusion Criteria

The inclusion criteria were patients aged more than 30 years and presence of at least 20 natural teeth. CP was defined as > 30% sites with a PPD ≥5mm that were positive for BOP and radiographic evidence of bone loss when evaluated using the long cone technique. The glycemic status of patients previously diagnosed with T2DM (having controlled T2DM since 5-10 year range) was confirmed by their HbA1C and random blood sugar level (RBS).

**Exclusion criteria**

1. Medical disorders such as cardiovascular or renal disease.
2. Malignancies, multiple sclerosis and
3. Tobacco chewers and smokers.
4. Pregnant, post-menopausal and lactating females.
5. History of antibiotic intake within 6 weeks.
6. History of any periodontal therapy within 6 months.

## Armamentarium (Color Plate IV)

Following material and armamentarium was used for the assessment of clinical parameters and for the collection of blood-

For examination of the patient:

1. Mouth mirror
2. UNC-15 (Hu-Freidy) periodontal probe.
3. Tweezer
4. Dental Explorer
5. Disposable gloves
6. Disposable face mask
7. Kidney tray
8. Weight measuring machine
9. Height measuring tape.
10. Waist circumference measuring tape.
11. Cotton swab.
12. Sphygmomanometer and stethoscope.

**For drawing blood**

1. Spirit
2. Sterilized cotton
3. 2 ml disposable syringe
4. 2 ml plastic vial
5. Tourniquet

**For collection of GCF sample (Color Plate V)**

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

## Assessment of periodontal and clinical parameters

**1. Probing pocket depth (PPD)**

It was measured using Hu Friedy UNC-15 periodontal probe on 4 sites of all present teeth. Patients were considered healthy if they exhibited probing depth < 3mm & there was no clinical attachment loss. Patients were diagnosed with CP if they exhibited PPD ≥ 5mm and CAL ≥ 5mm at multiple sites.

**2. Clinical attachment level (CAL)**

It was measured using Hu Friedy UNC-15 periodontal probe on 4 sites from the cementoenamel junction to the base of the pocket of all the present teeth. Patients were considered healthy if they exhibited no clinical attachment loss. Patients were diagnosed with chronic periodontitis if they exhibited clinical attachment level ≥ 5mm at multiple sites.

**3. Plaque index (PI) (Silness and Loe, 1964)**[56]

PI was examined in the scoring units of teeth: distofacial, facial, mesiofacial 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 Let 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. |

Totaling the scores around each tooth obtains the Plaque index score for the area. If the scores around each tooth are added and divided by four, the Plaque index score of each tooth is obtained.

$$\textbf{Plaque index (PI)} = \frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$$

The following suggested nominal scale was used for patient evaluation.

| Scores | Rating |
|---|---|
| 0 | Excellent |
| 0.1-0.9 | Good |
| 1.0- 1.9 | Fair |
| 2.0- 3.0 | Poor |

**4. Gingival index (GI) (Loe and Silness, 1963)**[57]

This is a system 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 Let 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 |

Totaling the scores around each tooth obtains the Gingival index score for the area. If the scores around each tooth are added and divided by four, the Gingival index score of each tooth is obtained.

$$\textbf{Gingival index (GI)} = \frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$$

The numerical score of the Gingival index taken into consideration for varying degrees of clinical gingivitis were as follows-

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

**5. Measurement of Body Mass Index (BMI)**

The body mass index is a measure of relative weight based on an individual's mass and height. The BMI is used in a wide variety of contexts as a simple method to assess how much an individual's body weight departs from what is normal or desirable for a person of his or her height.

Parameters used to measure BMI were height in meters and weight in kilograms. Weighing machine was used to measure weight and measuring tape was used to measure height.

**Calculations:**

$$\textbf{BMI} = \frac{\textbf{Weight (kg)}}{\textbf{Height (m}^2\textbf{)}}$$

Classification of BMI according to WHO:

Normal = 18.50-24.99 $kg/m^2$

Overweight = 25.00-29.99 $kg/m^2$

Obese = ≥30 $kg/m^2$

**6. Measurement of Waist circumference (WC)**

Central obesity is assessed by measuring Waist circumference. To measure WC, measuring tape was used. The patient was asked to stand straight and starting at the top of hip bone the measuring tape was taken all the way around, leveled with belly button, making sure that it's not too tight and that it's straight. The readings were obtained in centimeters (cm).

Normal values of WC:

Males – 90cm

Females – 80cm.

## Laboratory armamentarium for assessment of biochemical parameters

- Calibrated, volumetric transfer pipettes with 0-5 µl range, 5-50 µl range, 50-200 µl range and 200-1000 µl range
- Sterilized test tubes with test tube stand
- Distilled water
- Beakers, measuring cylinder
- Sterile gloves

**Laboratory equipment (Color Plate VI)**

- -80°C deep freezer (REMI EquipmentsPvt. Ltd.)
- Lab Centrifuge machine (R-8C, REMI EquipmentsPvt. Ltd.)
- Vortex mixer (CM 101, REMI EquipmentsPvt. Ltd.)
- ELISA reader (LISA Microplate reader, REMI EquipmentsPvt. Ltd.

## Assessment of Biochemical parameters

### Site selection and GCF collection:

Only one site per patient was selected on day 1 as a sampling site in periodontitis groups (Group II & III), whereas in healthy Group multiple sites (3-5 sites per patients) with an absence of inflammation were sampled to ensure the collection of an adequate amount of GCF.

In patient with CP, the site showing the greatest CAL and signs of inflammation was selected for sampling. GCF was collected by placing the micro capillary pipette at the entrance of gingival sulcus and gently touching the gingival margin. A standardized volume was collected using calibration on white colour-coated 1-5µl calibrated volumetric micro-capillary pipettes.

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.

### Blood and Serum sample collection

A total of 2ml of blood was collected from antecubital fossa by venipuncture using a 20 gauge needle. The blood sample was allowed to clot at room temperature and after 1 hour, serum was separated from blood by centrifuging at 1500 g for 10 minutes.

### Evaluation of Leptin from serum and GCF (Color Plate VI)

Samples were assayed for leptin levels using commercially available ELISA (Enzyme linked immune-sorbent assay) Kinesis Dx Human Leptin (LEP) ELISA Kit. Samples

were analyzed according to the instruction manual at the Department of Biochemistry. Briefly GCF and serum samples were diluted with dilution buffer in the kit and the amount of leptin was determined. All samples have been run in duplication.

**Reagents**

1. Microtiter Coated Plate (96 wells) – 1 no

2. Human LEP Biotin Conjugated Detection Antibody, 1 ml – 1 vial

3. Standard 64ng/ml – 0.5 ml

4. Streptavidin:HRP Conjugate - 6 ml

5. Wash Buffer (30X) – 20 ml

6. Standard Diluent – 3 ml

7. Substrate A – 6 ml

8. Substrate B – 6 ml

9. Stop Solution – 6 ml

**Additional materials required**

1. Microplate reader capable of measuring absorbance at 450 nm.

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

3.100 ml and 1 liter graduated cylinders.

4. Absorbent paper.

5. Distilled or deionized water.

6. Wash bottle or automated microplate washer.

7. Log-log graph paper or computer and software for ELISA data analysis.

8. Tubes to prepare standard or sample dilutions.

## Reagent preparation for both serum and GCF samples

1. All reagents and samples are brought to room temperature (18 - 25ºC) before use.

2. To make 1X Wash Solution, add 10 ml of 30X Wash Buffer in 290 ml of DI water.

3. For standard preparation: a vial of 120µl Original Standard was briefly spun to which 120µl Standard diluent was added to prepare a 32 ng/ml standard. The solution was thoroughly dissolved by a gentle mix. Pipetting of 120µl standard into each tube was done. The stock standard solution was used to produce a dilution series (shown below). Each tube was thoroughly mixed before the next transfer.

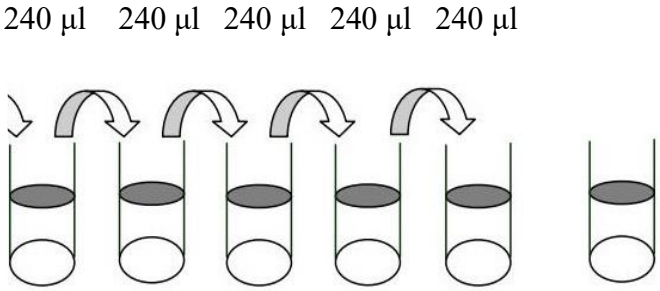



**Figure 1: Preparation of Standard for Leptin Assay**

| | | **Std5** | **Std4** | **Std3** | **Std2** | **Std1** |
|---|---|---|---|---|---|---|
| **Diluent Volume** | Original Standard +Standard diluent | 240 µl | 240 µl | 240 µl | 240 µl | 240 µl |
| **Conc.** | 64ng/ml | 32ng/ml | 16ng/ml | 8ng/ml | 4ng/ml | 2ng/ml |

## Assay procedure

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use.
2. Remove the number of strips required for the assay.
3. Pipette out 50 µl of Standards and 40 µl Samples into the respective wells as mentioned in the work list. Note do not add the sample, Biotin Conjugate and Streptavidin-HRP to the blank well.
4. Pipette out 10 µl of Biotin Conjugate into each sample well. Do not pipette into the blank and standards wells.
5. Pipette out 50 µl of Streptavidin-HRP Conjugate into each sample and standards well. Do not pipette into the Blank well.
6. Cover the plate and incubate for 1 hour at 37 ℃ in the incubator.
7. Aspirate and wash plate 4 times with 1X Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
8. Then add Substrate A 50 µl, then Substrate B 50 µl to each well including Blank well. Gently mixed, incubate for 10 min at 37 ℃ in dark.
9. Pipette out 50 µl of Stop Solution. Wells should turn from blue to yellow in colour.
10. Read the absorbance at 450 nm within 15 minutes after adding the Stop Solution blanking on the zero standards.

## Assay Procedure Summary

Bring all reagents and samples to room temperature (18 - 25ºC) before use.

↓

Pipette out 50 µl of Standards and 40 µl Samples into the respective wells

↓

Pipette out 10 µl of Biotin Conjugate into each sample well.

↓

Pipette out 50 µl of Streptavidin-HRP Conjugate into each sample and standards well.

↓

Cover the plate and incubate for 1 hour at 37 °C in the incubator.

↓

Aspirate and wash plate 4 times with 1X Wash Buffer.

↓

Add Substrate A 50 µl to each well.

↓

Add Substrate B 50 µl to each well.

↓

Gently mixed, incubate for 10 min at 37 ℃ in dark.

↓

Pipette out 50 µl of Stop Solution.

↓

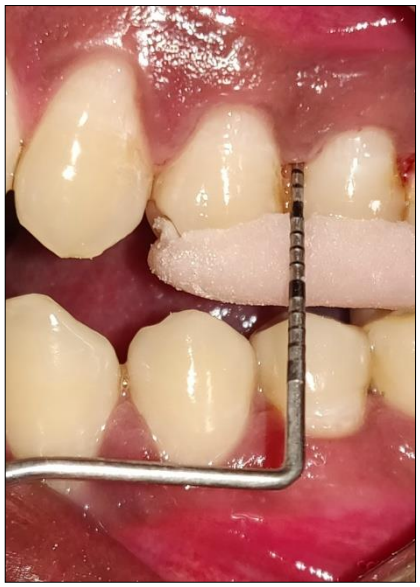
Read the absorbance at 450 nm within 15 minutes after adding the Stop Solution

blanking on the zero standards.

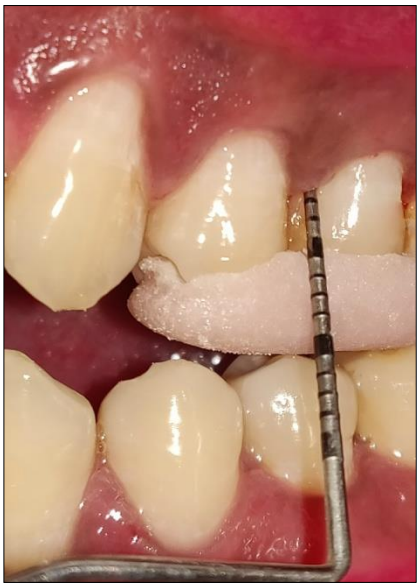
.

# Color Plate I

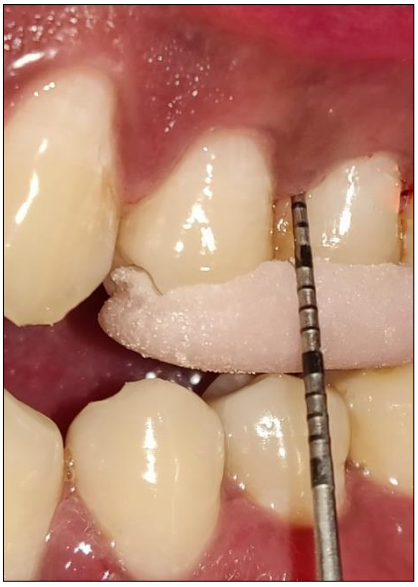
## Group I (Healthy Periodontium)



**Probing depth at baseline in Group I**



**Probing depth at 3 months in Group I**

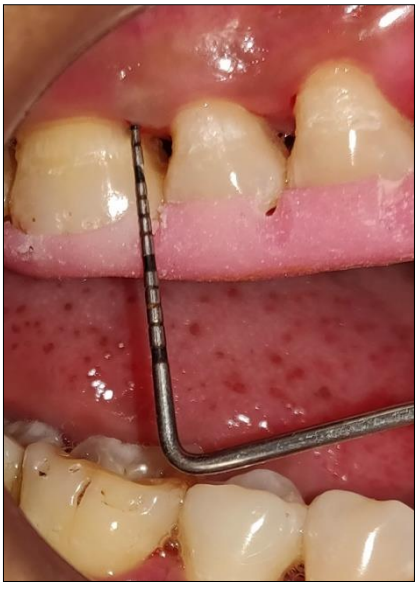


**Probing depth at 6 months in Group I**

# Color Plate II
# Group II (Chronic Periodontitis)

**Probing depth at baseline in Group II**

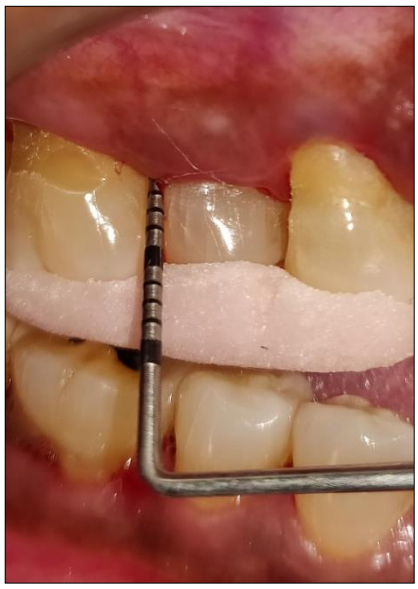
**Probing depth at 3 months in Group II**



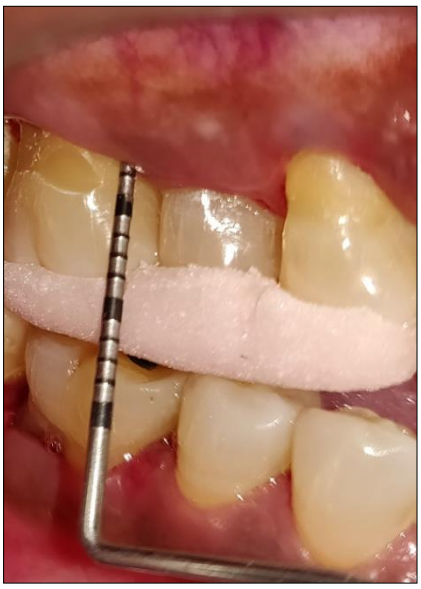
**Probing depth at 6 months in Group II**

# Color Plate III

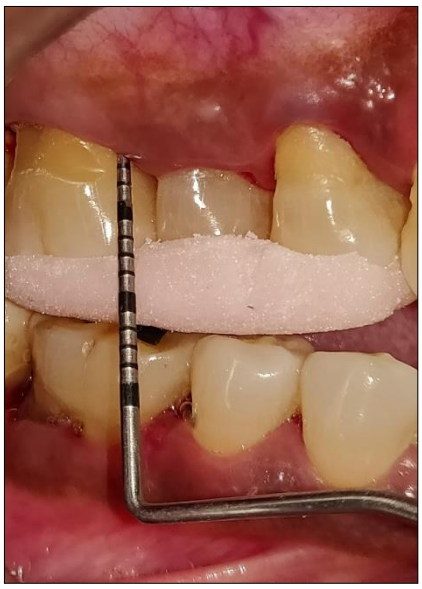
## Group III (Chronic Periodontitis with Type 2 Diabetes Mellitus)



**Probing depth at baseline in Group III**

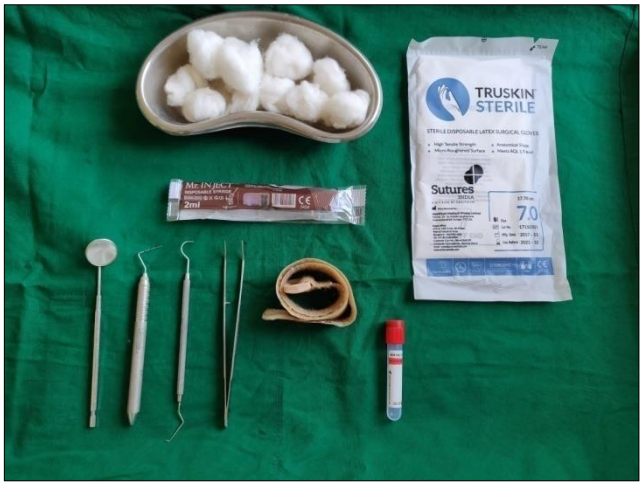


**Probing depth at 3 months in Group III**

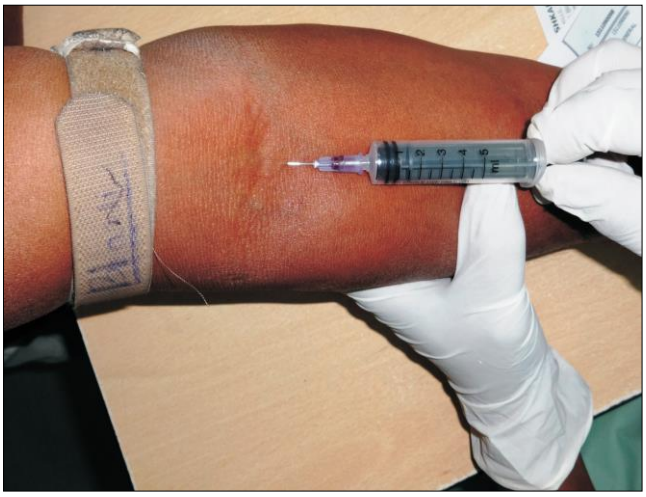


**Probing depth at 6 months in Group III**

# Color Plate IV



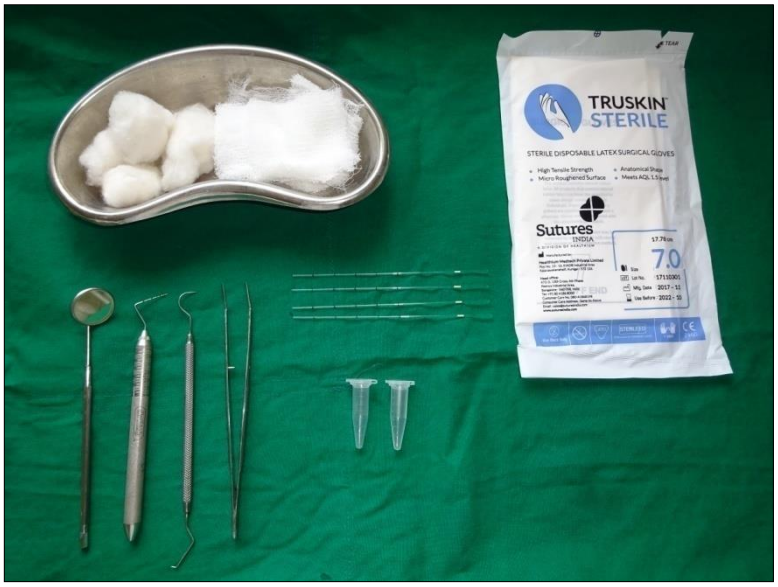

**Armamentarium for clinical examination and serum collection**



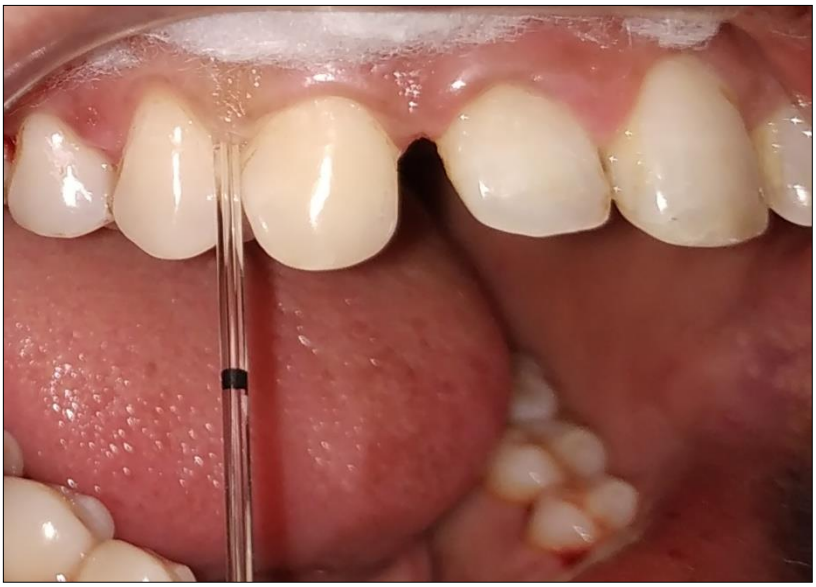
**Collection of blood**

**Collected serum sample**

# Color Plate V




**Armamentarium for clinical examination and GCF collection**



**GCF Collection**

# Color Plate VI

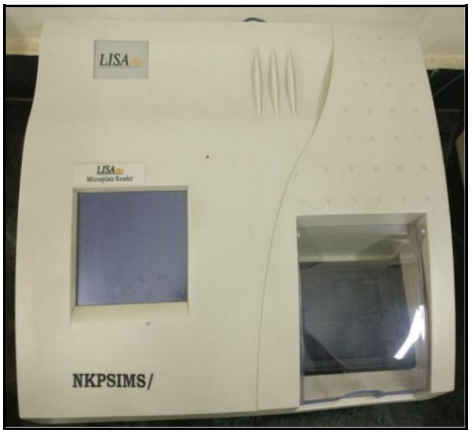
**Deep Freezer**

**Kinesis Dx Human Leptin ELISA Kit**


**Centrifuge Machine**


**Vortex Mixer**

**Micro-plate Washer**




**Micro-plate Reader**

# RESULTS

The aim of the present experimental study was to evaluate the levels of leptin in periodontally healthy, CP and CP with T2DM patients. Leptin is one of the recently introduced biomarker, of the various biomarkers found until now. Since leptin is a well known adipocytokine, we conducted the present study to hypothesize the link between leptin and chronic periodontitis. Also, since this biomarker has a role in insulin resistance, we further evaluated whether leptin levels are higher in DM patients with chronic periodontitis. In addition, since adipose tissue has been found to secrete immuno modulatory molecules such as adipokines, association of leptin with obese patients was also observed in our study.

+

For this research we had recruited patients which were examined clinically and biochemically and then categorized into three groups. Leptin levels were estimated using ELISA in GCF and serum samples to evaluate and compare their levels at baseline and after non surgical periodontal therapy.

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

**Statistical analysis**

The periodontal parameters were summarized in terms of mean and standard deviation and the difference of means was compared using one-way analysis of variance (ANOVA). The comparison of these parameters across times was performed using repeated measure ANOVA. The analysis was performed independently in each group. Further, at each time point, the comparison of parameters was performed across groups using one-way ANOVA followed by Tukey's post-hoc comparison. The change in the parameters at 3 and 6 months with reference to baseline was tested for statistical significance using paired t-test. The change was also assessed at 3 and 6 months across groups using one-way ANOVA followed by Tukey's post-hoc comparison. The anthropometric parameters like BMI and waist circumference were compared across groups using ANOVA followed by Tukey's post-hoc comparison. The pre and post comparison of leptin levels in serum as well as GCF were compared using paired t-test. Also, the pre levels of serum leptin were compared across groups using one-way ANOVA followed by Tukey's post-hoc test. The analysis was repeated for post levels of leptin. Likewise, analysis was performed for GCF leptin levels. The change in the leptin levels of serum and GCF were compared in each group using paired t-test.

Further, the comparison of change was also performed across groups using one-way ANOVA followed by Tukey's post-hoc test. Serum and GCF leptin levels were also compared across BMI categories in each group using one-way ANOVA followed by Tukey's test. Also, the comparison of HbA1c and RBS were carried out between baseline and 6 months using paired t-test. The pre and post comparison of these parameters across groups was performed using one-way ANOVA followed by Tukey's post-hoc test. The change in HbA1c and RBS values across groups was tested for significance of difference using one-way ANOVA. All the analyses were performed using SPSS ver 20.0 (IBM Corp) and the statistical significance was tested at 5% level.

**Clinical Parameters at baseline**

As per our requirement of classifying the patients into three different groups clinical parameters were assessed which included PI, GI, PPD and CAL. Significant differences among three groups were observed using one-way ANOVA. The difference of mean clinical attachment levels between Group II and III was evaluated using independent sample t-test. Tukey's post-hoc test for pair wise comparison reveals significant difference between Groups I and III, as well as Groups I and II as indicated by different superscripts ("a", "b" and "c") in Table 1. Same superscript on the means of different groups depict that the means of groups does not differ statistically from each other whereas different superscript on the means of different groups depict that there is statistically significant difference between the groups.

**Table 1 (Graph 1)** provides the descriptive statistics for various periodontal parameters in three groups. The mean PI at baseline in Group I was 0.38 ± 0.28, while it was 2.09 ±

0.40 for Group II and 2.45 ± 0.33 for Group III. The difference in the means across groups was statistically highly significant with p-value < 0.0001.

The mean GI at baseline was found to be 0.08 ± 0.07 for Group I, 1.83 ± 0.30 for Group II and 2.05 ± 0.40 or Group III respectively. The difference in the means across groups was statistically highly significant with p-value < 0.0001. The mean PPD at baseline was highest for Group III (5.6 ± 0.38mm) than Group II (4.98 ± 0.49mm) and was least for Group I (1.26 ± 0.19 mm). The difference in the means across groups was statistically highly significant with p-value < 0.0001.

The mean CAL was also highest in Group III (6.01 ± 0.38 mm) than Group II (5.35 ± 0.55 mm). The difference in the means across groups was statistically highly significant with p-value < 0.0001.

**Clinical parameters at 3 and 6 months in Group I, Group II and Group III.**

**Table 2(Graphs 2,3,4)** provide the intra-group comparison of clinical parameters across time points in each group. Repeated measures ANOVA with Greenhouse-Geisser correction was used to determine the significance of difference of measurements across times in each group. The mean PI and GI in Group I was 0.38 ± 0.28 and 0.08 ± 0.07 respectively which reduced to 0 at 3 months and was constant at 6 months. The mean PPD in Group I was 1.26 ± 0.19 mm at baseline which reduced to 0.50 ± 0.20 mm at 3 months and 0.23 ± 0.15mm at 6 months.

The mean PI in Group II was 2.09 ± 0.40 at baseline and reduced to 1.36 ± 0.33at 3 months and 0.87 ± 0.42 at 6 months. In Group III the mean PI reduced from 2.45 ± 0.33

at baseline to 0.60 ± 0.19 at 3 months and 0.24 ± 0.20at 6 months. The mean GI in

Group II at baseline was 1.83 ± 0.30 and reduced to 0.80 ± 0.35 at 3 months and 0.49 ± 0.29 at 6 months. The GI for Group III was 2.05 ± 0.40 at baseline, while the values were 1.04 ± 0.38 at 3 months and 0.60 ± 0.35at 6 months (p-value < 0.0001).

The mean PPD at baseline in Group II was 4.98 ± 0.49 mm which reduced significantly to 3.57 ± 0.63 mm at 3 months and 2.42 ± 0.72 mm at 6 months (p-value < 0.0001). In Group III, the mean PPD value at baseline was found to be 5.60 ± 0.38 mm which reduced significantly to 4.20 ± 0.51 mm at 3 months and 3.07 ± 0.60 mm at 6 months and the reduction was statistically significant as indicated by p-value < 0.0001.

Similarly, the mean clinical attachment level (CAL) in Groups II was 5.35 ± 0.55 mm at baseline, 3.94 ± 0.68 mm at 3 months and 2.81 ± 0.78 mm at 6 months. Whereas in Group III, the mean CAL at baseline was 6.01 ± 0.38 mm , 4.77 ± 0.64 mm at 3 months and 3.75 ± 0.86 at 6 months. The mean CAL was highly significantly different across time points as indicated by p value < 0.0001.

**Mean change in clinical parameters at 3 and 6 months with reference to baseline after non surgical periodontal therapy**

All the clinical parameters were assessed again at 3 and 6 months post therapy and in our results a significant reduction was found in means of PI, GI, PPD and CAL after a comprehensive non surgical periodontal therapy. Repeated measures ANOVA with Greenhouse-Geisser correction was used to determine the significance of difference of measurements across times in each group. Post treatment at 3 and 6 months, the mean changes in PI for Group I was found to be 0.38 ± 0.28, which remained constant at 6 months. For Group II, the mean change in PI was 0.73 ± 0.49 at 3 months and 1.22 ±

0.50 at 6 months. For Group III, the mean change was 1.85 ± 0.14 at 3 months and 2.21 ± 0.13 at 6 months.

While the mean change at 3 and 6 months post treatment GI for Group I was 0.08 ± 0.07 and remained constant at 6 months. The values for Group II were 1.03 ± 0.05 at 3 months and 1.34 ± 0.01 at 6 months. Group III showed mean change of 1.01 ± 0.02 at 3 months and 1.45 ± 0.05at 6 months.

The mean change in PPD at 3 and 6 months post treatment was found to be highest for Group II(1.42 ± 0.15 mm at 3 months and 2.57 ± 0.26 mm at 6 months) than for Group III(1.40 ± 0.14 mm at 3 months and 2.53 ± 0.24 mm at 6 months) and least for Group I (0.76 ± 0.01 mm at 3 months and 1.03 ± 0.04 mm at 6 months).

While mean gain in CAL at 3 and 6 months post treatment was 0 for Group I, and significantly higher for Group II (1.41 ± 0.15 mm at 3 months and 2.55 ± 0.25 mm at 6 months) than Group III (1.23 ± 0.42 mm at 3 months and 2.25 ± 0.67 mm at 6 months) respectively. The difference in the means of all the parameters across groups was statistically highly significant with p-value < 0.0001. **Table 3 (Graphs 5,6,7)**

**Correlations of clinical parameters among Group I, Group II and Group III**

In Group I, the mean change in clinical parameters at 3 and 6 month showed same means and standard deviation respectively for GI and PI, while a reduction in PPD was found which was statistically significant. A significant reduction was found in clinical parameters among Group II and III. However an intergroup comparison was done to find the difference in reduction of inflammation based on the influence of systemic condition of patients.

**Table 4** provides the statistical comparison of periodontal parameters between all three groups at pre- treatment and 3 and 6 months post-treatment stages.

At baseline and at 3 and 6 months, the mean values of clinical parameters showed statistically significant difference across groups as indicated by p-values< 0.0001. Tukey's post-hoc test revealed that the mean value of all the parameters were significantly higher in Group III as compared to Group II. The difference in the mean clinical attachment levels between Group II and III was evaluated using independent sample t-test, which was statistically highly significant with a p-value < 0.0001.

At 3 and 6 months post treatment, the mean change in clinical parameters was **(Graph 8, 9)**:

In Group I- PI (0.38 ± 0.28), GI (0.08 ± 0.07), PPD (0.76 ± 0.01, 1.03 ± 0.04 mm ), CAL 0.00 mm.

In Group II-PI (0.73 ± 0.49, 1.22 ± 0.50), GI (1.03 ± 0.05, 1.34 ± 0.01), PPD (1.42 ± 0.15 mm, 2.57 ± 0.26 mm), CAL (1.41 ± 0.15 mm, 2.55 ± 0.25 mm)

In Group III- PI (1.85 ± 0.14, 2.21 ± 0.13 ), GI (1.01 ± 0.02 ,1.45 ± 0.05 ), PPD (1.40 ± 0.14 mm, 2.53 ± 0.24 mm), CAL (1.23 ± 0.42 mm, 2.25 ± 0.67)

PPD and CAL showed statistically significant reduction in Group II as compared to Group III as indicated by p-value < 0.0001. The difference in the means of remaining parameters between Group II and III was evaluated using independent sample t-test, which was statistically highly significant with p-value < 0.0001, except for GI, where the p-value was 0.0009 **(Table 4).**

**BMI and WC across study groups**

It has been stated in literature that adipose tissue is responsible for sustained inflammatory response and release of various cytokines. Adiposity leading to obesity may accentuate the release of pro-inflammatory cytokines. Thus BMI along with WC were found and correlated among three groups.

**Table 5 (Graph 10)** provides the statistical comparison of BMI and WC among three groups. The difference of mean BMI across groups was statistically significant as indicated by p-value of 0.0001.Analysis using one way ANOVA followed by Tukey's post-hoc comparison revealed that the mean was statistically significantly higher in Group III (25.67 ± 2.73 $kg/m^2$) compared to Group II (23.63 ± 4.16 $kg/m^2$) and Group I (22.00 ± 2.13 $kg/m^2$) and. The WC measures were categorized according to gender, where in the male category, the mean WC was higher in Group III (83.88 ± 4.94 cm) as compared to Group II (81.60 ± 10.17 cm) and Group I (80.57 ± 14.41 cm); however, the difference of means was statistically insignificant with p-value of 0.6601. On similar lines, in female category, the mean WC was higher in Group III as compared to other groups; however, the difference of means was statistically insignificant as revealed by p-value 0.4933.

**Leptin levels in serum and GCF in Group I, Group II and Group III at baseline and after non surgical periodontal therapy**

Leptin is considered to be an inflammatory marker whose levels are seen to be increased in association with chronic periodontitis state due to enhanced cytokine infiltration. The reduction in levels of leptin was supposed to be due to effective periodontal therapy. So to know the effectiveness of lowering the pathologic condition in each group associated with leptin levels SRP was done in all the patients.

**Table 6 (Graph 11)** shows the statistical comparison of pre and post levels of parameters in three groups.

The comparison of pre-treatment and post-treatment serum and GCF leptin level across three groups was also performed using one-way ANOVA. The mean pre-treatment serum leptin level in Group I was 2.46 ± 0.35 ng/ml, 7.82 ± 0.53 ng/ml in Group II and 13.41 ± 0.64 ng/ml in Group III . At post-treatment level, the mean serum leptin level decreased significantly in Group I (2.36 ± 0.33 ng/ml), Group II (6.57 ± 0.60 ng/ml) and Group III (12.15 ± 1.27 ng/ml). For pre-treatment, the mean serum leptin levels across three study groups differed highly significantly with p-value < 0.0001. Same was the observation for serum leptin levels at post-treatment.

As regard to GCF leptin level, at pre-treatment level, the mean value was in Group I was 7.28 ± 0.55 ng/µl, 5.19 ± 0.61 ng/µl for Group II and 3.97 ± 0.70 ng/µl for Group III. At post-treatment level, the mean GCF leptin level increased statistically significantly in Group I (7.98 ± 0.51) as well as in Group II (6.34 ± 0.67) and Group III (5.00 ± 0.78). The mean GCF leptin level, pre and post-treatment was highly significantly with p-value< 0.0001.

**Leptin levels in serum and GCF at baseline and after non surgical periodontal therapy among Group I, II and III**

T2DM is itself an immunosuppressive condition which favors the destruction of periodontal tissues as compared to non diabetic patients. So, the study evaluated the effects of SRP over leptin levels and comparison of these levels enabled to assess the reduction in different study groups.

Tukey's post-hoc test for the intergroup comparison of pre-treatment and post-treatment serum and GCF leptin level in three groups for serum leptin level revealed that the mean pre-treatment serum leptin level in Group III (13.41 ± 0.64 ng/ml) was significantly higher as compared to Group II (7.82 ± 0.53 ng/ml) and Group I (2.46 ± 0.35 ng/ml). At

post-treatment level, the mean serum leptin level decreased significantly in Group III (12.15 ± 1.27 ng/ml) as well as Group II (6.57 ± 0.60 ng/ml) and Group I (2.36 ± 0.33 ng/ml).

The mean serum leptin levels at pre and post treatment in all groups differed highly significantly with p-value < 0.0001. Similar was the observation in Group II and III.

While for GCF leptin level, at pre-treatment level, the mean value was statistically significantly higher in Group I (7.28 ± 0.55 ng/µl) as compared to Group II (5.19 ± 0.61 ng/µl) and Group III (3.97 ± 0.70 ng/µl). At post-treatment level, the mean GCF leptin level increased statistically significantly in Group I (7.98 ± 0.51) as compared to Group II (6.34 ± 0.67) and Group III (5.00 ± 0.78). The mean GCF leptin level at pre and post-treatment was highly significantly with p-value< 0.0001. **(Table 7)**

**BMI and leptin levels in GCF and serum**

As it has been stated in studies obesity is one of the risk factors for enhancement of an inflammatory state. To find out whether leptin is also linked with obesity, patients were categorized into three groups i.e. ≤ 24.9 (Normal), 25-29.9 (Overweight), ≥ 30 (Obese) and varying levels of leptin were evaluated using paired t-test.

**Table 8 (Graph 12)** gives the statistical comparison of mean change of GCF and serum leptin levels between pre and post-treatment stages as per BMI categories. The mean pre and post treatment serum leptin level was found to be highest in obese category (11.77 ± 3.07, 9.63 ± 3.31) than overweight (11.07 ± 4.06, 10.17 ± 3.84) and normal weight category (6.81 ± 4.23, 6.06 ± 3.80).

The pre and post treatment GCF leptin levels were lowest in obese category (3.53 ± 0.73, 4.43 ± 0.85) than overweight category (4.11 ± 1.26, 5.09 ± 1.18) and were found to be highest in normal weight category (5.97 ± 1.26, 6.94 ± 1.08).

In the normal and over-weight categories, the mean serum leptin levels were significantly higher at pre-treatment as compared to post-treatment,with p-values < 0.0001. In obese category, the mean leptin level was higher at pre-treatment as compared to post-treatment, but the difference was statistically insignificant with a p-value 0.1295.The mean GCF leptin levels at post treatment were statistically higher than pre-treatment with a p-value< 0.0001 for each BMI category.

**RBS and HbA1C in Group I, Group II and Group III**

HbA1C was considered to be confirmatory test to validate the diabetic condition of the patient along with RBS test.

**Table 9 (Graph 13, 14)** provides the statistical comparison of RBS and $HbA_1C$ levels among three groups.

The comparison of pre-treatment and post-treatment HbA1c and RBS across three study groups was performed using one-way ANOVA separately. The mean HbA1c at baseline and post treatment in Group I was 4.95 ± 0.35 % and 4.83 ± 0.35 % respectively. For Group II, the HbA1c was 5.23 ± 0.44% and 5.14 ± 0.42% respectively and it was 6.87 ± 0.75% and 6.71 ± 0.76% respectively for Group III.

The mean RBS values at baseline and post treatment were 98.40 ± 8.45 mg/dl and 96.03 ± 6.90 mg/dl respectively for Group I, 107.97 ± 10.51mg/dl and 103.27 ± 8.63 mg/dl respectively for Group II and 178.27 ± 73.69 mg/dl and 156.33 ± 44.07 mg/dl respectively for Group III.

The difference in the means across groups was statistically highly significant with p-value < 0.0001.

**RBS and HbA1C values after non surgical periodontal therapy**

To evaluate the effect of non surgical periodontal therapy on glycaemic status RBS and HbA1c levels were assessed again after 6 months post therapy. A significant reduction was observed in both the values in all three groups. **Table 10** provide the statistical comparison of RBS and HbA1c values between three groups.

The paired analysis using Tukey's test revealed that the mean RBS value at baseline and post treatment was significantly higher in Group III in (178.27 ± 73.69 mg/dl and 156.33 ± 44.07 mg/dl) as compared to Group II (107.97 ± 10.51 mg/dl, 103.27 ± 8.63 mg/dl) and Group I (98.40 ± 8.45 mg/dl, 96.03 ± 6.90 mg/dl) and the results were highly significant with p<0.0001.

Similarly the mean HbA1c value at baseline and post treatment was highest in Group III (6.87 ± 0.75 % and6.71 ± 0.76 % ) as compared to Group II (5.23 ± 0.44 % and 5.14 ± 0.42 % ) and Group I(4.95 ± 0.35 % and 4.83 ± 0.35 %), the results being significant with p<0.0001.

# DISCUSSION

Periodontitis and diabetes mellitus are common, chronic and multifactorial disorders affecting a wide range of population. Inflammation is the central feature of both disorders and inflammatory processes is up regulated in periodontal tissues of diabetic patients. The effect of accumulated AGE on cell to matrix, matrix to matrix interactions, increased tissue oxidative stress, altered endothelial cell function and elevated activity of matrix metalloproteinases in periodontal tissues aggravate the severity of periodontal diseases.[58]On the other hand, the increased production of pro- inflammatory cytokines in periodontitis and their spill over from local periodontal tissues have been suggested to participate in the β- cell damage in diabetic patients.[59] Thus, periodontitis accompanied with hyperglycemic state becomes a critical issue with regards to oral health.

Periodontal infection has been known to induce systemic immune response. The possible mechanism by which periodontitis may provoke systemic inflammatory response is through the inflamed and ulcerated pocket epithelium which forms an easy port of entry for oral micro organisms. Lipopolysaccharide (LPS), a potent bacterial endotoxin can be disseminated throughout the body originating from periodontal lesion. Also, the locally produced pro inflammatory mediators such as IL-1, IL-6, TNF-α etc are dumped into systemic circulation and cause their effect on distant organ systems.[60]

A substantial amount of evidence is available in the literature, establishing a link between obesity, diabetes mellitus and periodontitis. Obesity is considered both as a direct and indirect risk for progression of periodontitis. Indirectly it affects glycaemic control, while the secretion of pro inflammatory cytokines by adipocytes makes obesity a direct risk factor. Over accumulation of fatty acids in adipose tissue leads to hypertrophy of adipocytes which increases the production of reactive oxygen species (ROS), thus exaggerating inflammation of adipocytes eventually leading to increased expression of pro inflammatory cytokines. This mechanism exacerbates periodontal inflammation. Increased levels of circulating FFA, ROS and adipokines disrupt insulin signaling leading to β- cell dysfunction, systemic insulin resistance and eventually onset of diabetes mellitus. Adipose tissue is no longer considered merely a depository of fat containing cells, but an active endocrine organ with adipocytes and many other cells, capable of releasing hormones and cytokines which are also known as adipokines. Adipokines when released into systemic circulation have the ability to mediate and modulate inflammatory activity.[61]

Leptin, the best known adipokine which was discovered in 1994, is derived from the Greek word “LEPTOS” meaning thin. The gene (ob gene) is located on chromosome

7q31 DNA. The receptors belong to cytokine receptor class 1 family and are highly expressed in hypothalamus, T lymphocytes and vascular endothelial cells. Circulating leptin crosses blood brain barrier and mediates its action through the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway (JAK–STAT3) pathway. The activation of phosphoinositide 3 kinase (PI3K) in the hypothalamus is related to the ability of leptin to inhibit feeding. Leptin receptor-mediated JAK–STAT signaling is essential for regulation of food intake and body weight, while leptin-stimulated PI3K signaling appears to be important for regulation of glucose metabolism. Studies in obese rodents suggested that leptin resistance is associated with impairment of leptin transport across the blood– brain-barrier (BBB), reduction of leptin-mediated JAK–STAT signaling, and induction of suppressor of cytokine signaling-3 (SOCS-3). Attenuation of leptin sensitivity in the brain leads to excess triglyceride accumulation in adipose tissue, as well as muscle, liver, and pancreas, resulting in impaired insulin sensitivity and secretion.[62]

Leptin is now considered as a link between neuro endocrine and immune system. Certain adipokines can enhance a fore mentioned inflammation and, in that context, the modulation of innate immunity by leptin has been well-established. Leptin acts as a stimulatory mediator on neutrophils, as it activates chemotaxis and oxidative function. Also, Leptin promotes activation and proliferation of circulating monocytes and induces the production of pro inflammatory cytokines by monocytes (such as IL-1, TNF or IL-6) and in synergy with lipopolysaccharide (LPS), it stimulates the oxidative burst and enhances chemotactic responses by acting as a chemo attractant in monocytes or macrophages. On the other hand proinflammatory cytokines secreted in response to inflammatory condition like periodontitis also increase leptin synthesis.[63]A positive

correlation of serum leptin concentration and periodontal inflammation has been found in literature, conversely it is known that leptin is present within healthy gingiva and its concentration declines with the increase in severity of the gingival inflammation and periodontal pocket formation.[18]

Thus, leptin is an inflammatory marker which is found to have an association with DM and inflammatory conditions as well as obesity. So the present study was planned with a hypothesis that leptin levels in serum of patients with generalized chronic periodontitis with and without type II diabetes mellitus are elevated due to high inflammatory burden which gets reduced after phase I periodontal therapy, and if the GCF leptin levels are declined as a result of high inflammatory burden and increase in their levels after phase I periodontal therapy.

The present case control study was conducted on 90 patients.

Group I- Control group (30 periodontally and systemically healthy patients)

Group II- Test group (30 patients with chronic periodontitis)

Group III- Test group (30 patients with chronic periodontitis and Type II diabetes mellitus)

Having three groups in our study helped us to better evaluate the levels of leptin according periodontal disease severity in association with diabetes.

In present study, in order to evaluate the effect of non-surgical periodontal therapy on GCF and serum leptin levels, patients in all the groups were treated with non-surgical approach (SRP). Clinical parameters recorded were PI, GI, PPD and CAL at baseline and at 3 and 6 months after phase I therapy. GCF and serum leptin samples were collected and assessed using **Kinesis Dx Human Leptin (LEP)** ELISA kit for GCF and serum **(Krishgen Biosystems)** at baseline and after 6 months. To assess the effect of

SRP on glycaemic status, HbA1c and RBS values were evaluated at baseline and at 6 months.

There was a significant improvement in clinical parameters such as PI, GI, PPD and CAL after non-surgical periodontal therapy in all the groups but the Group II showed better improvement as compared to Group III. The levels of serum leptin were found to be greater, while that of GCF was lesser in diabetic patients with chronic periodontitis when compared to that of patients with chronic periodontitis without DM. Levels of serum leptin decreased after Phase I therapy in the groups but the reduction was higher in Group II as compared to Group III. GCF leptin levels increased after Phase I therapy but the increase was higher in Group II as compared to Group III. HbA1c and RBS values also decreased in CP patients with T2DM.

**Clinical Parameters at baseline and after non surgical periodontal therapy**

At baseline the mean PI was highest in Group III (2.45 ± 0.33) as compared to Group II (2.09 ± 0.40) and Group I (0.38 ± 0.28). Similarly, the mean GI value was found to be 0.08 ± 0.07 in Group I, 1.83 ± 0.30 in Group II and 2.05 ± 0.40 in Group III. At baseline the mean PPD was 5.6 ± 0.38 mm, which was highest in Group III, followed by 4.98 ± 0.49 mm and 1.26 ± 0.19 mm in Group II and Group I respectively. The mean CAL also was highest in Group III (6.01 ± 0.38 mm) than in Group II (5.35 ± 0.55 mm), whereas it was 0.00 mm in Group I.

The difference in periodontal parameters such as PI, GI, PPD and CAL across the three study groups was found to be statistically significant.

This indicates that the selection of patients that is the inclusion criteria for the study was strictly adhered to. Mean values of all the periodontal parameters were statistically

increased in Group II (chronic periodontitis) and Group III (chronic periodontitis with type II diabetes mellitus) as compared to (periodontally healthy patients).

To evaluate and compare the effectiveness of SRP in resolution of inflammation, we determined the means of clinical parameters at baseline and after 3 and 6 months of non surgical periodontal therapy.

The mean change in PPD at 3 and 6 months post treatment was highest for Group II (1.42 ± 0.15 mm and 2.57 ± 0.26 mm respectively) followed by Group III (1.40 ± 0.14 mm and 2.53 ± 0.24 mm respectively) and Group I (0.76 ± 0.01 mm and 1.03 ± 0.04 mm respectively). While mean change in CAL at 3 and 6 months post treatment was also highest for Group II (1.41 ± 0.15 mm and 2.55 ± 0.25 mm respectively) than Group III (1.23 ± 0.42 mm and 2.25 ± 0.67 mm respectively) was 0 mm for Group I. The difference in the means of all the parameters across groups was statistically highly significant with p-value < 0.0001. Group II showed better response to non-surgical periodontal therapy as compared to Group III in terms of PPD and CAL.

Similar results were found in a study conducted by **Badersten A et al. (1981)**[64] in which the effect of non surgical periodontal therapy was assessed in patients with chronic periodontitis and showed reduction of PPD, CAL, PI and GI in at 4 months. This proved the effectiveness of non surgical periodontal therapy in reducing inflammation in patients with chronic periodontitis.

**Correlations of clinical parameters among Group II and Group III**

It is reported in many studies that DM patients propose a risk for initiation of inflammation due to hyperglycemic state as well as more collagen destruction. Above all in DM healing of tissues is also delayed due to accumulated glycation end products.

So it was probable to find lesser improvement in clinical parameter after therapy as compared to absence of DM.

Although clinical parameters i.e. PI, GI, PPD and CAL showed statistically significant reduction after therapy in both Group II and Group III, the overall reduction of inflammation in Group III was not found as per the reduction in Group II. A study done by **Rode PA et al. (2018)**[65] also found better improvement in clinical parameters in patients with chronic periodontitis than in patients with CP and T2DM. Since patients with T2DM pose a risk for the initiation of inflammation, it is probable to find lesser improvement in clinical parameters after therapy as compared to those with absence of DM.

Similarly, **K V S et al. (2014)**[66] monitored the effect of SRP on clinical parameters and glycaemic control in patients with type 2 diabetes mellitus by estimating the HbA1c and GCF TNF-α levels and demonstrated a significant improvement in periodontal status following periodontal treatment. A reduction in TNF-α level and the HbA1c values was also observed. Mechanical non-surgical periodontal therapy was effective in reducing periodontal inflammation and the concentration of circulating cytokines (TNF-α), which in turn helped to improve the metabolic control in type 2 Diabetes Mellitus.

**BMI and WC across study groups**

Obesity is an established risk factor for morbidity as well as mortality. Though BMI is the most common measure of obesity, it does not reflect body shape. BMI may be delusive in individuals with a high proportion of lean muscle mass. Waist circumference, a more accurate measure of the distribution of body fat, has been shown to be more strongly associated with morbidity and mortality. Nevertheless, despite the

American Heart Association's recent endorsement of both BMI and waist circumference as primary tools for assessing adiposity, waist circumference is less commonly used than the BMI in both research and clinical settings.[67] So the indicators for obesity measured in our study were both, BMI and WC. The mean BMI in Group I was 22.00 ± 2.13 kg/$m^2$, 23.63 ± 4.16 kg/$m^2$ in Group II and in Group III, 25.67 ± 2.73 kg/$m^2$. The explanation for the mean BMI values above the normal limits in the III Group can possibly be attributed to the presence of DM.

It is reported that obese patients often show increased lipid and blood glucose levels which in turn alters T-cell and macrophage response to infection, which leads to increased cytokine production, creating an imbalance in human immune system. This may increase the risk for infection, and this may explain the reported association of obesity with certain infectious diseases; periodontitis being one amongst them. The results in our study demonstrated a BMI of 25.67 ± 2.73 kg/$m^2$ (overweight category) in T2DM patients with chronic periodontitis which are similar to a study conducted by **Dalla Vecchia CF et al. (2005)**[68] who found association of periodontitis in overweight and obese patients. A positive correlation between the BMI index and the occurrence of periodontitis was observed with a significantly higher prevalence of periodontitis in obese than in normal weight females.

Obesity results in increased production of free fatty acids and apoptosis of β cells of pancreas causing insulin resistance. To find the relationship between periodontal disease, obesity, and insulin resistance, **Pham T.A.V et al. (2018)**[69] conducted a study and found that the prevalence of periodontitis in obese group and Type 2 diabetic group was significantly higher than those without these conditions. Patients with obesity or Type 2 diabetes had significantly greater pocket depth and clinical attachment loss than those who were not obese or diabetic. Multivariate logistic regression showed that the

likelihood for periodontitis was highest in the obese and Type 2 diabetic group, explaining a significant association between these three conditions.

This similar trend of increasing BMI with increase in severity of periodontal destruction was observed in our study. This finding can be supported by the explanation that insulin resistance appears to mediate the relationship between obesity and periodontal disease.

The second indicator of obesity assessed in the study was Waist Circumference (WC). The mean value of WC for males in Group I, Group II and Group III was 81.60 ± 10.17 cm, 80.57 ± 14.41 cm and 83.88 ± 4.94 cm respectively, while for females, the WC was 78.75 ± 8.37 cm, 77.81 ± 12.30 cm and 81.85 ± 5.98 cm respectively. The comparison of WC across the three study groups showed an increasing trend in WC, with highest mean value of WC in Group III (chronic periodontitis with T2DM) but the difference was found to be statistically insignificant.

**Leptin levels in GCF and serum in each group at baseline and after non surgical periodontal therapy**

Studies have showed that SRP works efficiently in reducing the inflammation, thus decreasing serum leptin levels and increasing GCF leptin levels. In our study, a reduction in serum leptin levels was observed. The serum leptin levels reduced from 2.46 ± 0.35 ng/ml at baseline to 2.36 ± 0.33 ng/ml 6 months post therapy in Group I, while the reductions were from 7.82 ± 0.53 ng/ml to 6.57 ± 0.60 ng/ml in Group II and from 13.41 ± 0.64 ng/ml to 12.15 ± 1.27 ng/ml in Group III respectively.

Serum leptin levels are known to be increased in periodontitis as leptin is a part of cytokine network that governs the inflammatory response. On the other hand GCF leptin levels are highest in disease free periodontium and the levels decrease with increasing periodontal destruction. The reason for increased GCF leptin levels in

healthy periodontium is that the clinically healthy tissue is ‘‘armed’’ with pro-inflammatory host components to aid in maintaining an infection-free periodontium.[46] Although the association between periodontitis and serum and GCF leptin levels has been studied in the literature, very few studies have evaluated the effect of SRP on serum and GCF leptin levels.

To evaluate the efficiency of non surgical periodontal therapy in reduction of serum leptin levels, **Kardesler L et al. (2010)**[34] assessed the effects of initial periodontal treatment on clinical periodontal measurements, glycemic control, and systemic inflammatory mediator levels in patients with type 2 diabetes and chronic periodontitis. Clinical periodontal parameters showed improvement at all time points, adiponectin levels increased in the systemically healthy group, while leptin levels decreased at 3 months in systemically healthy patients with CP, which was similar to the results of our study. **Shimada Y et al. (2010)**[47] also observed that serum leptin, IL-6, and CRP levels differed significantly between healthy and CP patients. The serum leptin level was associated with clinical parameters, mean alveolar bone loss, and BMI. Serum leptin, IL-6, and CRP levels decreased significantly after SRP, concluding that periodontal treatment is effective in reducing serum leptin, IL-6, and CRP levels. In our study also, we found a statistically significant reduction in leptin levels after SRP. This suggests that leptin could be mediating factor that connects metabolic syndrome and periodontitis.

The results of our study are contradictory to those reported by **Goncalves TE et al. (2015)** [39] in which the serum levels of leptin and adiponectin did not change in groups with and without obesity after therapy. The failure of SRP in reducing leptin levels could be attributed to the fact that the majority of the patients still retained several

residual pockets (PD ≥5 mm) after the proposed nonsurgical periodontal therapy which sustained the inflammatory state in patients.

For GCF leptin levels, the increase was from 7.28 ± 0.55 ng/µl to 7.98 ± 0.51 ng/µl at baseline and 6 month post therapy respectively in Group I, while the increase was from 5.19 ± 0.61 ng/µl to 6.34 ± 0.67 ng/µl in Group II and from 3.97 ± 0.70 ng/µl to 5.00 ± 0.78 ng/µl for Group III. The results of our study are in accordance with those conducted by **Karthikeyan BV et al (2007)**[46] who found that the highest mean leptin concentration in GCF was obtained for healthy group and the least for CP group. In contrast, the lowest serum leptin concentration was obtained for the healthy group, and the highest for CP group. This suggests a negative correlation of GCF leptin concentration and a positive correlation of serum leptin concentration to periodontal destruction. These results suggest that as the periodontal destruction progresses, the GCF leptin concentration decreases and the serum leptin concentration increases.

Another study conducted by **Goncalves TE et al. (2015)**[43] evaluated the effects of SRP on GCF and serum levels of adipokines in patients with chronic periodontitis with or without obesity. The results of our study were in accordance as SRP increased the GCF concentrations of TNF- α and leptin in patients with obesity. However, there were no changes in serum levels of any adipokines for any group after therapy. This finding was contradictory to our study where serum leptin levels decreased significantly after SRP. The reason for unchanged serum levels could be that in this study, several residual sites remained for both groups despite the initial and maintenance periodontal therapies, which could represent a bacterial load great enough to maintain systemic inflammation at baseline levels. The authors also stated that the unchanged serum levels of adipokines, despite some clinical improvements, may be related to the inherent

susceptibility of the patients to systemic inflammation, independently of periodontal infection or the obese state.

The results of our study showed a negative correlation between the GCF leptin concentration and periodontal disease progression which are in accordance with the study done by **Johnson RB et al. (2001)**[70], who also showed that GCF leptin concentration decreases with an increase in probing pocket depth. The higher concentration of GCF leptin levels in healthy periodontium could be protective to gingival tissues.

The decrease in GCF leptin levels with the increasing severity of periodontitis could be attributed to two mechanisms. The first mechanism explains that the expansion of vascular network caused by vascular endothelial growth factor, which possibly increases the net rate of leptin removal from the gingival tissue and could raise serum leptin levels during gingival inflammation. Thus, in addition to adipose tissue, gingiva could also be a source of circulating leptin in patients with periodontal disease. Secondly, it could be a body's defense mechanism to counteract periodontal inflammation as leptin is a part of the immune response and host defense mechanism.[71,72]

However, the results of our study regarding the effect of SRP on GCF leptin levels are contradictory the study done by **Meharwade VV et al. (2014)**[51] who evaluated the effect of SRP on GCF leptin levels in patients with chronic periodontitis and found no significant difference in the GCF leptin levels among the study group sites. The authors hypothesized that the presence of various leptin receptors and soluble leptin receptor (SLR) in periodontal tissues may be responsible for the obtained inconsistent results.

**Leptin levels in GCF and serum at baseline and after non surgical periodontal therapy among Group I, II and III**

In our study, when serum leptin levels at baseline was compared among three groups, Group III was found to have highest value and least was with Group I, the difference being highly significant ($p< 0.0001$). When serum leptin levels after treatment were compared among three groups, Group III was found to have higher value than Group II and least was with Group I the difference was highly significant ($p< 0.0001$).

It is suggested that resistance to leptin in β-cells might prevent the inhibitory effect of leptin on insulin secretion resulting in hyperinsulinemia, which might exhaust pancreatic β-cells leading to development of T2DM.[35]

The results of our study are in accordance with the study conducted by **Diwan AG et al. (2018)[45]** who analyzed the serum levels of adiponectin and leptin in the participants with type 2 diabetes mellitus (T2DM) and obesity and their correlation with hypertension and dyslipidemia. Leptin levels were significantly higher in diabetics as compared to non diabetics in both males and females. Adiponectin levels were significantly higher in diabetics than in non diabetic participants irrespective of gender. The authors concluded that adiponectin and leptin levels may be used as important clinical markers for T2DM and obesity.

Another study done by **Chen HD et al. (2017)**[44] evaluated the relationship between serum leptin concentration and metabolic syndrome (MetS) and obesity in T2DM patients. Fasting glucose level, HbA1c, insulin level, HOMA-IR, and leptin level were higher in DM patients who had MetS. Moreover, higher serum leptin levels were significantly correlated with BMI levels in DM patients. Thus, a higher serum leptin level was found to be positively associated with MetS in T2DM patients.

**AL-Azawy VS et al. (2014)**[52] also conducted a study to evaluate the serum level of leptin and adiponectin in periodontitis with and without T2DM and to determine the association between levels of biochemical markers in serum. The results of our study were consistent to this study as there was a significant elevation in mean level of leptin in periodontitis group and periodontitis+T2DM group in comparison to that in healthy control, moreover, the comparison between two groups of patients showed that the mean level of leptin was increased in periodontitis+T2DM group.

The results of our study are in contradiction to the study done by **Mohammadzadeh G et al.(2013)**[36] who aimed to evaluate variations in serum leptin levels in non obese patients with T2DM**.** The serum leptin level in T2DM patients was significantly lower than that in non- diabetic patients but serum leptin level was strongly and positively correlated with BMI. The authors speculated that the reduction in serum leptin levels in type 2 diabetes and even lower levels in patients with poorly controlled diabetes was likely due to male gender, insulin deficiency, and defect of β-cell function.

Another study that did not show significant difference in leptin levels in diabetic patients was done by **Tatti P et al. (2001)**.[73] The authors compared the leptin concentrations and its relationship with some anthropometric and blood parameters related to insulin resistance in a population of moderately obese T2DM patients. The leptin levels were lower in the diabetic population only when both sexes were combined and were higher in the females of both groups. Among the non diabetics, the leptin levels appeared to be related to BMI, while this was not the case in the diabetics. Insulin has been shown to increase plasma leptin concentration and the patients recruited in the

study showed a borderline insulin levels. This seems to indicate the insignificant correlation of leptin concentration in DM patients.

Our results are inconsistent to a study conducted by **Haffner SM et al. (1996)**[33] who demonstrated that the leptin concentrations were not different in diabetics and non-diabetics and that the association of leptin with the components of obesity, principally the BMI, was similar in diabetic and non diabetic subject. The authors in the study measured the leptin levels at the same time at which the diagnosis of diabetes mellitus was made. Thus, Haffner's cohort was notably represented by newly diagnosed patients with a younger age group, which might be the reason for insignificant results.

**BMI and leptin levels in GCF and serum**

It has been suggested that leptin levels are increased in presence of increased inflammatory cytokine levels. Obesity is also considered a risk factor for ingression of inflammation due to release of various adipocytokines and pro-inflammatory cytokines. To confirm the association of leptin with obesity all patients were categorized into three groups according to the WHO criteria and correlation was assessed.

In patients with BMI $\leq$ 24.9 kg/m$^2$ (N=68) the serum leptin levels were reduced from 6.81 ± 4.23 ng/ml to 6.06 ± 3.80 ng/ml, while GCF leptin levels increased from 5.97 ± 1.26 ng/µl to 6.94 ± 1.08 ng/µl.

Similar trend of reduction in serum and increase in GCF leptin levels was observed in patients with BMI 25-29.9 kg/m$^2$ (Overweight) (N=16) where serum leptin levels were reduced from 11.07 ± 4.06 84 ng/ml to 10.17 ± 3.84 ng/ml, and increased in GCF from 4.11 ± 1.26 ng/µl to 5.09 ± 1.18 ng/µl.

In patients with BMI ≥ 30 (Obese) (N=6) serum leptin levels were reduced from 11.77 ± 3.07 ng/ml to 9.63 ± 3.31 ng/ml and increased in GCF from 3.53 ± 0.73 ng/µl to 4.43 ± 0.85 ng/µl.

Adipocytes secrete a number of molecules such as TNF-α, leptin and free fatty acids that can influence the ability of the body to respond to insulin and metabolize glucose. So, increased size of adipose tissues directly correlates with an increase in inflammatory markers through increased adipocytes. In our study we found positive correlation of obesity with increased levels of leptin which is also an adipocytokine. The results of our study are in accordance with a study conducted by **Altay U et al. (2013)**[37] who evaluated the short-term changes in systemic inflammatory, lipid, and glucose parameters in the presence of obesity after periodontal treatment. The periodontal parameters, anthropometric measurements, and serum lipid levels, hs- CRP, fasting blood glucose, insulin, IL-6, TNF-α, and leptin were measured before and 3 months after non-surgical periodontal treatment. A decrease in serum TNF-α and IL-6 levels and leptin scores in individuals with obesity was observed. Thus, concluding that non-surgical periodontal treatment causes a decrease in the levels of some circulating proinflammatory cytokines and may be associated with a decrease in insulin resistance in the obese population.

**Zimmermann GS et al. (2013)**[38] evaluated the local and circulating levels of adipocytokines in serum and GCF of individuals who are obese and individuals who are normal weight (NW) with CP. The normal weight patients without periodontitis presented the lowest serum leptin levels. Serum levels of IL-6 and leptin were correlated with obese patients with CP group. Individuals in the obese group presented the highest

concentration of leptin. Thus, periodontitis mainly influenced the circulating levels of resistin and adiponectin, whereas both obesity and periodontitis affected the circulating levels of leptin in favor of pro inflammation.

**RBS and HbA1C values after non surgical periodontal therapy**

It is well known that the reduction in periodontal inflammation can improve insulin sensitivity, and it can act as an adjunct for better glycemic control, or at least delay the burden of diabetic complications. So, to evaluate the effect of SRP on glycaemic status, HbA1c and RBS levels were determined before and after non surgical periodontal therapy.

A significant reduction was observed in both the values in all three groups. The mean RBS value in Group III reduced from 178.27 ± 73.69 mg/dl to 156.33 ± 44.07 mg/dl post therapy. The mean HbA1c value in Group III was 6.87 ± 0.75 % which reduced to 6.71 ± 0.76 % post treatment and the results were significant.

The beneficial effect of periodontal treatment on HbA1c levels has been widely studied in many systematic reviews. Poorly controlled diabetic patients show a fast decrease in HbA1c level after SRP as compared to well-controlled diabetic patients. It is seen that HbA1c levels rebound to baseline levels. The health status of these patients is associated with complications, as they are prone to infections. So, they might require a change in medications or diet. Therefore, well controlled patients were evaluated in this study.[74]

Studies have shown that insulin resistance is increased in the diabetic patient under the influence of local factors, and hence decreases the healing response of tissues. Hence, it can be hypothesized that, after non surgical periodontal therapy, local factors are removed, leading to a decrease in insulin resistance with a decrease in proinflammatory

cytokines (e.g., TNF-α, IL-6) associated with local factors, thus improving insulin sensitivity, ultimately leading to better glycemic control, and in turn, promoting a better wound healing capacity of tissues in T2DM patients.[75]

Our results are similar to those reported by **Goel K et al. (2017)**[74] who evaluated and compared the effect on glycemic control and periodontal status with or without nonsurgical periodontal therapy in patients with T2DM and CP and found a significant reduction in HbA1c levels after 3 months in the group subjected to SRP than control group, suggesting that nonsurgical periodontal therapy had beneficial effect on HbA1c level in moderately controlled type 2 diabetic patients.

Another study done by **Mauri-Obradors E et al. (2018)**[76] evaluated the effect of non-surgical periodontal treatment on serum HbA1c levels in patients with type 2 diabetes and concluded that SRP significantly improved the periodontal and metabolic parameters in patients treated with SRP, whereas in the control group no improvement was observed. Thus, non-surgical periodontal treatment resulted in a better glycaemic status of type 2 diabetes patients and demonstrated the importance of oral health in their general health.

However, the results of our study are inconsistent to a study done by **Kapellas K et al. (2017)**[77] who reported the effect of periodontal therapy on glycaemic control among people with obesity and found that periodontal therapy did not significantly reduce HbA1c and CRP or periodontal status at 3 months. The reason being, maintenance of oral hygiene during the study was not reinforced, and consequently, gingival bleeding levels remained consistent with baseline measures. The authors assumed that residual periodontal pocketing and its concomitant inflammation perpetuated chronic inflammation systemically thus leading to no change in HbA1c and CRP.

A study conducted by **Engebretson SP et al. (2013)**[27] also evaluated if non-surgical periodontal treatment could reduce HbA1c in persons with T2DM and moderate to advanced chronic periodontitis. Our results were in contradiction as the study showed that non-surgical periodontal therapy did not improve glycemic control in patients with DM and moderate to advanced chronic periodontitis. The population in this study was overweight/obese (average BMI of 35). These results suggest that obesity and uncontrolled HbA1c limit the effect of periodontal treatment on the reduction of systemic inflammation. On the other hand, in our study, the patients were moderately overweight, which is considered normal in diabetic patients and could be attributed to improvement in glycaemic status.

This study plays a crucial role in demonstrating the effect of non surgical periodontal therapy in reduction of periodontal inflammation and metabolic parameters in healthy patients, patients with chronic periodontitis and chronic periodontitis with Type 2 DM and also in evaluating the GCF and serum leptin levels in all three groups and their levels after non surgical periodontal therapy.

# CONCLUSION

The present study was undertaken to evaluate the effects of non-surgical periodontal therapy on GCF and serum leptin levels in periodontally healthy, chronic periodontitis and chronic periodontitis with type 2 diabetes mellitus patients and whether leptin levels are associated and found to be increased in chronic periodontitis with and without type 2 diabetes mellitus. A total of 90 patients were recruited and categorized into 3 groups, with 30 patients in each. Group I being healthy, Group II being patients with chronic periodontitis and Group III being patients with chronic periodontitis with T2DM. All the patients were assessed clinically and biochemically for categorization into respective groups. Clinical parameters evaluated were PI, GI, PPD and CAL at baseline and at 3 and 6 months of non surgical periodontal therapy. Biochemical parameters included were RBS and HbA1C levels to assess the diabetic status of patients and to evaluate

changes in glycaemic status after 6 months of non surgical periodontal therapy. Other parameters were BMI and WC to identify obesity. ELISA test was used to analyze leptin levels in GCF and serum at baseline and after 6 months of non surgical periodontal therapy.

There was a gradual increase in the severity of clinical parameters from Group I to Group II and significantly higher difference was observed among Group II and III and they were found to be positively correlated with increased levels of leptin in serum, while a negative correlation with GCF leptin levels was observed. A higher range of HbA1c and RBS levels was found in Group III. Non surgical periodontal therapy was effective in improving the clinical parameters in Group II and Group III. Also, a reduction in serum leptin levels and an increase in GCF leptin levels was observed. SRP also improved the glycaemic status of patients with T2DM.

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

1. A positive and significant association was observed between serum leptin levels and a negative association of GCF leptin levels in chronic periodontitis and chronic periodontitis with T2DM.
2. There exists a positive and significant association between reduction in inflammation along with reduction in serum leptin levels and increase in GCF leptin levels after non surgical periodontal therapy in chronic periodontitis and chronic periodontitis with T2DM.
3. There exists a positive and significant association between reduction of HbA1c and RBS after non surgical periodontal therapy in chronic periodontitis with T2DM.

4. A positive and significant association was observed between leptin levels in serum and a negative association of GCF leptin levels with obesity in chronic periodontitis and chronic periodontitis with T2DM.

Within the limits of our study, it can be concluded that in patients with chronic periodontitis and chronic periodontitis with T2DM, there may be an increase in serum leptin levels and decrease in GCF leptin levels with an increase in severity of periodontal destruction, which may further aggravate the periodontal destruction. Non surgical periodontal therapy improved glycaemic control in patients with chronic periodontitis and chronic periodontitis with T2DM, and also decreased the circulating levels of serum leptin and increased GCF leptin levels suggesting the possible role of leptin in mediating immune response.

There are few limitations of the study:

1. A handful of studies have evaluated the effect of SRP on GCF leptin levels. Further long-term multicenter trials are necessary to confirm the findings of the present study.

2. A larger sample size is desirable for substantiation of the findings.

Further studies are required to establish the effects of leptin on diabetes alone without presence of chronic periodontitis and leptin levels in chronic periodontitis with radiographic bone changes in various periodontal diseases and systemic conditions. Leptin levels if recognized would lead to better understanding of its functions and would help in establishing a therapeutic protocol at an earlier stage thereby preventing the patient getting prone to inflammatory diseases.

# REFERENCES

1. **Graves D**. Cytokines that promote periodontal tissue destruction. **J Periodontol** 2008; 79: 1585-1591.
2. **Mattila KJ, Nieminen MS, Valtonen VV, Rasi VP, Kesaniemi YA, Syrjala SL et al**. Association between dental health and acute myocardial infarction. **BMJ** 1989; 298: 779-781.
3. **Mealey BL**. Periodontal disease and diabetes. A two-way street. **J Am Dent Assoc** 2006; 137: 26S-31S.
4. **Khader YS, Taani Q**. Periodontal diseases and the risk of preterm birth and low birth weight: A meta-analysis. **J Periodontol** 2005; 76: 161-165.
5. **Moghadam SA, Shirzaiy M, Risbaf S**. The Associations between Periodontitis and Respiratory Disease. **J Nepal Health Res Counc** 2017; 15: 1-6.

6. **Chang WP, Chang WC, Wu MS, Pai JT, Guo YC, Chen KC et al**. Population-based 5-year follow-up study in Taiwan of osteoporosis and risk of periodontitis. **J Periodontol** 2014; 85: e24-30.
7. **Suresh S, Mahendra J**. Multifactorial relationship of obesity and periodontal disease. **J Clin Diagn Res** 2014; 8: ZE01-ZE03.
8. **Joshi SR, Parikh RM**. India - diabetes capital of the world: Now heading towards hypertension. **J Assoc Physicians India**. 2007; 55: 323–324.
9. **Soskolne WA, Klinger A**. The relationship between periodontal diseases and diabetes: An overview. **Ann Periodontol** 2001; 6: 91-98.
10. **Nishimura F, Iwamoto Y, Mineshiba J, Shimizu A, Soga Y, Murayama Y**. Periodontal disease and diabetes mellitus: the role of tumor necrosis factor-alpha in a 2-way relationship. **J Periodontol** 2003 ;74: 97-102.
11. **Genco RJ, Grossi SG, Ho A, Nishimura F, Murayama Y**. A proposed model linking inflammation to obesity, diabetes, and periodontal infections. **J Periodontol** 2005; 76: 2075-2084.
12. **Greenberg AS, Obin MS**. Obesity and the role of adipose tissue in inflammation and metabolism. **Am J Clin Nutr** 2006; 83: 461S-465S.
13. **Carbone F, La Rocca C, Matarese G**. Immunological functions of leptin and adiponectin. **Biochimie** 2012;94: 2082-2088.
14. **Margetic S, Gazzola C, Pegg GG, Hill RA**. Leptin: a review of its peripheral actions and interactions. **Int J Obes Relat Metab Disord** 2002; 26: 1407-1433.
15. **Indhu K, Saravanan T**. Serum Leptin Levels in Type 2 Diabetes Mellitus with Chronic Complications. **IOSR-JDMS** 2017; 16: 22-24.

16. **Schultze SM, Hemmings BA, Niessen M, Tschopp O**. PI3K/AKT, MAPK and AMPK signalling: protein kinases in glucose homeostasis. **Expert Rev Mol Med** 2012; 14: e1-e21.
17. **Al-Suhaimi EA, Shehzad A**. Leptin, resistin and visfatin: The missing link between endocrine metabolic disorders and immunity. **Eur J Med Res** 2013; 18: 1-13.
18. **Gangadhar V, Ramesh A, Thomas B**. Correlation between leptin and the health of the gingiva: A predictor of medical risk. **Indian J Dent Res** 2011; 22: 537-441.
19. **Leira Y, Ameijeira P, Domínguez C, Leira R, Blanco J**. The role of leptin as a biomarker in the relationship between periodontitis and chronic migraine. **J Clin Periodontol 2017**; 44: 1208-1214.
20. **Katsiki N, Mikhailidis DP, Banach M**. Leptin, cardiovascular diseases and type 2 diabetes mellitus. **Acta Pharmacol Sin** 2018 ; 39: 1176-1188.
21. **Gomez-Banuelos E, Navarro-Hernandez RE, Corona-Meraz F, Madrigal-Ruiz PM, Martín-Marquez BT, Pizano-Martinez OE et al**. Serum leptin and serum leptin/serum leptin receptor ratio imbalance in obese rheumatoid arthritis patients positive for anti-cyclic citrullinated peptide antibodies. **Arthritis Res Ther** 2015; 17: 1-9.
22. **Da Cruz GA, De Toledo S, Sallum EA, Sallum AW, Ambrosano GM, De Cassia Orlandi Sardi J et al**. Clinical and laboratory evaluations of non-surgical periodontal treatment in subjects with diabetes mellitus. **J Periodontol 2008**; 79:1150-1157.
23. **Sun WL, Chen LL, Zhang SZ, Wu YM, Ren YZ, Qin GM**. Inflammatory cytokines, adiponectin, insulin resistance and metabolic control after periodontal

intervention in patients with type 2 diabetes and chronic periodontitis. **Intern Med** 2011; 50: 1569-1574.

24. **Rajhans NS, Kohad RM, Chaudhari VG, Mhaske NH**. A clinical study of the relationship between diabetes mellitus and periodontal disease. **J Indian Soc Periodontol** 2011;15: 388-392.
25. **Modi CS, Desai SJ**. The effect of improved periodontal health on metabolic control in type 2 diabetes mellitus. **Adv Hum Biol** 2013; 3:21-28.
26. **Monea A, Mezei T, Monea M**. The influence of diabetes mellitus on periodontal tissues: A histological study. **Rom J Morphol Embryol** 2012; 53:491-495.
27. **Engebretson SP, Hyman LG, Michalowicz BS, Schoenfeld ER, Gelato MC, Hou W et al**. The effect of nonsurgical periodontal therapy on hemoglobin A1c levels in persons with type 2 diabetes and chronic periodontitis: A randomized clinical trial. **JAMA** 2013; 310:2523-2532.
28. **Perayil J, Suresh N, Fenol A, Vyloppillil R, Bhaskar A, Menon S**. Comparison of glycated hemoglobin levels in individuals without diabetes and with and without periodontitis before and after non-surgical periodontal therapy. **J Periodontol** 2014; 85: 1658-1666.
29. **Michalowicz BS, Hyman L, Hou W, Oates TW Jr, Reddy M, Paquette DW et al**. Factors associated with the clinical response to nonsurgical periodontal therapy in people with type 2 diabetes mellitus. **J Am Dent Assoc** 2014; 145: 1227-1239.
30. **Kaur PK, Narula SC, Rajput R, K Sharma R, Tewari S**. Periodontal and glycemic effects of nonsurgical periodontal therapy in patients with type 2 diabetes stratified by baseline HbA1c. **J Oral Sci** 2015; 57: 201-211.

31. **Quintero AJ, Chaparro A, Quirynen M, Ramirez V, Prieto D, Morales H et al**. Effect of two periodontal treatment modalities in patients with uncontrolled type 2 diabetes mellitus: A randomized clinical trial. **J Clin Periodontol** 2018; 45: 1098-1106.
32. **Myllymäki V, Saxlin T, Knuuttila M, Rajala U, Keinanen-Kiukaanniemi S, Anttila S et al**. Association between periodontal condition and the development of type 2 diabetes mellitus - results from a 15-year follow-up study. **J Clin Periodontol** 2018; 45:1276-1286.
33. **Haffner SM, Stern MP, Miettinen H, Wei M, Gingerich RL**. Leptin concentrations in diabetic and nondiabetic Mexican-Americans. **Diabetes** 1996;45: 822-824.
34. **Kardesler L, Buduneli N, Cetinkalp S, Kinane DF**. Adipokines and inflammatory mediators after initial periodontal treatment in patients with type 2 diabetes and chronic periodontitis. **J Periodontol** 2010; 81: 24-33.
35. **Bandaru P, Shankar A**. Association between plasma leptin levels and diabetes mellitus. **Metab Syndr Relat Disord** 2011; 9: 19-23.
36. **Mohammadzadeh G, Zarghami N**. Serum leptin level is reduced in non-obese subjects with type 2 diabetes. **Int J Endocrinol Metab** 2013; 11: 3-10.
37. **Altay U, Gurgan CA, Agbaht K**. Changes in inflammatory and metabolic parameters after periodontal treatment in patients with and without obesity. **J Periodontol** 2013; 84: 13-23.
38. **Zimmermann GS, Bastos MF, Dias Gonçalves TE, Chambrone L, Duarte PM**. Local and circulating levels of adipocytokines in obese and normal weight individuals with chronic periodontitis. **J Periodontol** 2013;84: 624-633.

39. **Goncalves TE, Feres M, Zimmermann GS, Faveri M, Figueiredo LC, Braga PG et al.** Effects of scaling and root planing on clinical response and serum levels of adipocytokines in patients with obesity and chronic periodontitis. **J Periodontol** 2015; 86: 53-61.
40. **Jing Ling X, Xin MH, Lu H, Xian E W, Lin Z**. Serum ratio of leptin to adiponectin in patients with chronic periodontitis and type 2 diabetes mellitus. **ISRN Biomarkers** 2014; 12:1-5.
41. **Ghallab NA, Amr EM, Shaker OG**. Expression of leptin and visfatin in gingival tissues of chronic periodontitis with and without type 2 diabetes mellitus: A study using enzyme-linked immunosorbent assay and real-time polymerase chain reaction. **J Periodontol** 2015; 86: 882-889.
42. **Mendoza-Azpur G, Castro C, Pena L, Guerrero ME, De La Rosa M, Mendes C et al.** Adiponectin, leptin and TNF-α serum levels in obese and normal weight Peruvian adults with and without chronic periodontitis. **J Clin Exp Dent** 2015; 7: e380-e386.
43. **Goncalves TE, Zimmermann GS, Figueiredo LC, Souza Mde C, Da Cruz DF, Bastos MF et al.** Local and serum levels of adipokines in patients with obesity after periodontal therapy: One-year follow-up. **J Clin Periodontol** 2015; 42: 431-439.
44. **Chen HD, Wu DA, Hou JS, Subeq YM, Li JC, Hsu BG**. Positive correlation of serum leptin levels with obesity and metabolic syndrome in patients with type 2 diabetes mellitus. **Int J Clin Exp Pathol** 2017; 10: 4852-4859.
45. **Diwan AG, Kuvalekar AA, Dharamsi S, Vora AM, Nikam VA, Ghadge AA**. Correlation of serum adiponectin and leptin levels in obesity and type 2 diabetes mellitus. **Indian J Endocrinol Metab** 2018; 22:93-99.

46. **Karthikeyan BV, Pradeep AR**. Gingival crevicular fluid and serum leptin: their relationship to periodontal health and disease. **J Clin Periodontol** 2007; 34: 467–472.
47. **Shimada Y, Komatsu Y, Ikezawa-Suzuki I, Tai H, Sugita N, Yoshie H**. The effect of periodontal treatment on serum leptin, interleukin-6, and C-reactive protein. **J Periodontol** 2010; 81: 1118-1123.
48. **Vadvadgi VH, Saini R, Padmawar N**. An evaluation and correlation of leptin in gingival crevicular fluid and serum in health, gingivitis and periodontitis. **Int J Experiment Dent Sci** 2012; 1: 93-97.
49. **Ay ZY, Sutcu R, Kocak H, Kirzioglu FY**. Serum leptin levels and their proportional relationship with pro-and antiinflammatory mediators in aggressive periodontitis: Preliminary report. **Turk J Med Sci** 2013; 43: 825-830.
50. **Gundala R, Chava VK, Ramalingam K**. Association of leptin in periodontitis and acute myocardial infarction. **J Periodontol** 2014 ; 85: 917-924.
51. **Meharwade VV, Gayathri GV, Mehta DS**. Effects of scaling and root planing with or without a local drug delivery system on the gingival crevicular fluid leptin level in chronic periodontitis patients: a clinico-biochemical study. **J Periodontal Implant Sci** 2014; 44: 118-125.
52. **Al-Azawy VS, Al-Ghurabi BH, Sh.Jabir A**. Evaluation of Serum Leptin and Adiponectin Levels in Iraqi Patients with Chronic Periodontitis. **IOSR-JDMS** 2015; 14:63-67.
53. **Selvarajan S, Perumalsamy R, Emmadi P, Thiagarajan R, Namasivayam A**. Association between gingival crevicular fluid leptin levels and periodontal status - A biochemical study on Indian patients. **J Clin Diagn Res** 2015; 9: ZC48-C53.

54. **Purwar P, Khan MA, Mahdi AA, Pandey S, Singh B, Dixit J et al**. Salivary and serum leptin concentrations in patients with chronic periodontitis. **J Periodontol** 2015; 86: 588-594.
55. **Khorsand A, Bayani M, Yaghobee S, Torabi S, Kharrazifard MJ, Mohammadnejhad F**. Evaluation of salivary leptin levels in healthy subjects and patients with advanced periodontitis. **J Dent Tehran** 2016; 13: 1-9.
56. **Silness J, Löe H**. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. **ActaOdontol Scand** 1964;22:121-135.
57. **Löe H, Silness J**. Periodontal disease in pregnancy I. Prevalence and severity. **ActaOdontol Scand** 1963;21: 533-551.
58. **Stanko P, Izakovicova Holla L**. Bidirectional association between diabetes mellitus and inflammatory periodontal disease. A review. **Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub** 2014;15:35-38.
59. **Zhou X, Zhang W, Liu X, Zhang W, Li Y**. Interrelationship between diabetes and periodontitis: role of hyperlipidemia. **Arch Oral Biol** 2015;60: 667-674.
60. **Loos BG**. Systemic markers of inflammation in periodontitis. **J Periodontol** 2005; 76: 2106-2115.
61. **Levine RS**. Obesity, diabetes and periodontitis-A triangular relationship? **Br Dent J** 2013; 215: 35-39.
62. **Yadav A, Kataria MA, Saini V, Yadav A.** Role of leptin and adiponectin in insulin resistance. **Clin Chim Acta** 2013; 417: 80-84.
63. **Abella V, Scotece M, Conde J, Pino J, Gonzalez-Gay MA, Gomez-Reino JJ et al**. Leptin in the interplay of inflammation, metabolism and immune system disorders. **Nat Rev Rheumatol** 2017;13: 100-109.

64. **Badersten A, Nilvius R, Egelberg J**. Effect of nonsurgical periodontaltherapy. I- Moderately advanced periodontitis. **J Clin Periodontol** 1981: 8: 57-72.
65. **Rode PA, Kolte RA, Kolte AP, Purohit HJ, Ahuja CR**. Relevance of single-nucleotide polymorphism to the expression of resistin gene affecting serum and gingival crevicular fluid resistin levels in chronic periodontitis and type 2 diabetes mellitus: A randomized control clinical trial. **J Indian Soc Periodontol** 2018.doi: 10.4103/jisp.jisp_361_18 (Accepted for publication)
66. **K V S, A S, P L, N S, S M A, Bhat D, Mundinamane DB**. The effect of scaling and root planing on glycaemic control, periodontal status and gingival crevicular fluid TNF-α levels in an Indian population- To reveal the ambivalent link. **J Clin Diagn Res** 2014; 8: ZC22-ZC26.
67. **Dagan SS, Segev S, Novikov I, Dankner R**. Waist circumference vs body mass index in association with cardiorespiratory fitness in healthy men and women: A cross sectional analysis of 403 subjects. **Nutr J** 2013 15; 12:12.
68. **Dalla Vecchia CF, Susin C, Rösing CK, Oppermann RV, Albandar JM**. Overweight and obesity as risk indicators for periodontitis in adults. **J Periodontol** 2005; 76:1721-1728.
69. **Pham TAV, Tran TTP**. The interaction among obesity, Type 2 diabetes mellitus, and periodontitis in Vietnamese patients. **Clin Exp Dent Res** 2018; 4: 63-71.
70. **Johnson RB, Serio FG**. Leptin within healthy and diseased human gingiva. **J Periodontol** 2001;72: 1254-1257.
71. **Arnalich F, López J, Codoceo R, Jimnez M, Madero R, Montiel C**. Relationship of plasma leptin to plasma cytokines and human survivalin sepsis and septic shock. **J Infect Dis** 1999;180: 908-911.

72. **Bullon P, Morillo JM, Ramirez-Tortosa MC, Quiles JL, Newman HN, Battino M**. Metabolic syndrome and periodontitis: is oxidative stress a common link? **J Dent Res** 2009; 88: 503-518.
73. **Tatti P, Masselli L, Buonanno A, Di Mauro P, Strollo F**. Leptin levels in diabetic and nondiabetic subjects. **Endocrine 2001**; 15: 305-308.
74. **Goel K, Pradhan S, Bhattarai MD**. Effects of nonsurgical periodontal therapy in patients with moderately controlled type 2 diabetes mellitus and chronic periodontitis in Nepalese population. **Clin Cosmet Investig Dent** 2017; 9: 73-80.
75. **Mohan M, Jhingran R, Bains VK, Gupta V, Madan R, Rizvi I et al**. Impact of scaling and root planing on C-reactive protein levels in gingival crevicular fluid and serum in chronic periodontitis patients with or without diabetes mellitus. **J Periodontal Implant Sci** 2014 ;44:158-168.
76. **Mauri-Obradors E, Merlos A, Estrugo-Devesa A, Jane-Salas E, Lopez-Lopez J, Vinas M**. Benefits of non-surgical periodontal treatment in patients with type 2diabetes mellitus and chronic periodontitis: A randomized controlled trial. **J Clin Periodontol** 2018; 45: 345-353.
77. **Kapellas K, Mejia G, Bartold PM, Skilton MR, Maple-Brown LJ, Slade GD et al**. Periodontal therapy and glycaemic control among individuals with type 2 diabetes: reflections from the PerioCardio study. **Int J Dent Hyg** 2017;15:e42-e51.

## Tables

**Table 1**: **Comparison of clinical parameters at baseline across three study groups**

| Parameters | Group [Mean ± SD] | | | p-value* |
|---|---|---|---|---|
| | Group I (n=30) | Group II (n=30) | Group III (n=30) | |
| Gingival index | 0.08 ± 0.07[a] | 1.83 ± 0.30[b] | 2.05 ± 0.40[c] | < 0.0001 (HS) |
| Plaque index | 0.38 ± 0.28[a] | 2.09 ± 0.40[b] | 2.45 ± 0.33[c] | < 0.0001 (HS) |
| Probing pocket depth (in mm) | 1.26 ± 0.19[a] | 4.98 ± 0.49[b] | 5.6 ± 0.38[c] | < 0.0001 (HS) |
| Clinical attachment level (in mm) | 0 ± 0[a] | 5.35 ± 0.55[b] | 6.01 ± 0.38[c] | < 0.0001 (HS) |

HS: Highly Significant

**Table 2: Intra-group comparison of clinical parameters across time points in each group.**

| Groups | Parameters | Time point [Mean ± SD] | | | p-value* |
|---|---|---|---|---|---|
| | | Baseline | 3 month | 6 month | |
| Group I (n=30) | Gingival index | 0.08 ± 0.07 | 0.00 ± 0.00 | 0.00 ± 0.00 | - |
| | Plaque index | 0.38 ± 0.28 | 0.00 ± 0.00 | 0.00 ± 0.00 | - |
| | Probing pocket depth (in mm) | 1.26 ± 0.19 | 0.50 ± 0.20 | 0.23 ± 0.15 | < 0.0001 (HS) |
| | Clinical attachment level (in mm) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | - |
| Group II (n=30) | Gingival index | 1.83 ± 0.30 | 0.80 ± 0.35 | 0.49 ± 0.29 | < 0.0001 (HS) |
| | Plaque index | 2.09 ± 0.40 | 1.36 ± 0.33 | 0.87 ± 0.42 | < 0.0001 (HS) |
| | Probing pocket depth (in mm) | 4.98 ± 0.49[a] | 3.57 ± 0.63[b] | 2.42 ± 0.72[c] | < 0.0001 (HS) |
| | Clinical attachment level (in mm) | 5.35 ± 0.55[a] | 3.94 ± 0.68[b] | 2.81 ± 0.78[c] | < 0.0001 (HS) |
| Group III (n=30) | Gingival index | 2.05 ± 0.40 | 1.04 ± 0.38 | 0.60 ± 0.35 | < 0.0001 (HS) |
| | Plaque index | 2.45 ± 0.33 | 0.60 ± 0.19 | 0.24 ± 0.20 | < 0.0001 (HS) |
| | Probing pocket depth (in mm) | 5.60 ± 0.38[a] | 4.20 ± 0.51[b] | 3.07 ± 0.60[c] | < 0.0001 (HS) |
| | Clinical attachment level (in mm) | 6.01 ± 0.38[a] | 4.77 ± 0.64[b] | 3.75 ± 0.86[c] | < 0.0001 (HS) |

HS: Highly Significant

**Table 3: Intra-group comparison of mean change in clinical parameters at 3 and 6 months in three study groups.**

| Groups | Parameters | Change in parameter [Mean ± SD] | | p-value |
|---|---|---|---|---|
| | | Baseline – 3 month | Baseline – 6 month | |
| Group I (n=30) | Gingival index | 0.08 ± 0.07 | 0.08 ± 0.07 | - |
| | Plaque index | 0.38 ± 0.28 | 0.38 ± 0.28 | - |
| | Probing pocket depth (in mm) | 0.76 ± 0.01 | 1.03 ± 0.04 | < 0.0001 (HS) |
| | Clinical attachment level (in mm) | 0.00 ± 0.00 | 0.00 ± 0.00 | - |
| Group II (n=30) | Gingival index | 1.03 ± 0.05 | 1.34 ± 0.01 | < 0.0001 (HS) |
| | Plaque index | 0.73 ± 0.49 | 1.22 ± 0.50 | 0.0043 (S) |
| | Probing pocket depth (in mm) | 1.42 ± 0.15 | 2.57 ± 0.26 | < 0.0001 (HS) |
| | Clinical attachment level (in mm) | 1.41 ± 0.15 | 2.55 ± 0.25 | < 0.0001 (HS) |
| Group III (n=30) | Gingival index | 1.01 ± 0.02 | 1.45 ± 0.05 | < 0.0001 (HS) |
| | Plaque index | 1.85 ± 0.14 | 2.21 ± 0.13 | < 0.0001 (HS) |
| | Probing pocket depth (in mm) | 1.40 ± 0.14 | 2.53 ± 0.24 | < 0.0001 (HS) |
| | Clinical attachment level (in mm) | 1.23 ± 0.42 | 2.25 ± 0.67 | < 0.0001 (HS) |

HS: Highly Significant; S: Significant

**Table 4: Inter-group comparison of mean clinical parameters at three time points**

| Time-point | Parameters | Group [Mean ± SD] | | | p-value |
|---|---|---|---|---|---|
| | | Group I (n=30) | Group II (n=30) | Group III (n=30) | |
| Baseline | Gingival index | 0.08 ± 0.07[a] | 1.83 ± 0.30[b] | 2.05 ± 0.40[c] | < 0.0001 (HS)* |
| | Plaque index | 0.38 ± 0.28[a] | 2.09 ± 0.40[b] | 2.45 ± 0.33[c] | < 0.0001 (HS)* |
| | Probing pocket depth (in mm) | 1.26 ± 0.19[a] | 4.98 ± 0.49[b] | 5.60 ± 0.38[c] | < 0.0001 (HS)* |
| | Clinical attachment level (in mm) | 0.00 ± 0.00 | 5.35 ± 0.55 | 6.01 ± 0.38 | < 0.0001 (HS) † |
| 3 months | Gingival index | 0.00 ± 0.00 | 0.80 ± 0.35 | 1.04 ± 0.38 | 0.0009 (S)† |
| | Plaque index | 0.00 ± 0.00 | 1.36 ± 0.33 | 0.60 ± 0.19 | < 0.0001 (HS)† |
| | Probing pocket depth (in mm) | 0.50 ± 0.20[a] | 3.57 ± 0.63[b] | 4.20 ± 0.51[c] | < 0.0001 (HS)* |
| | Clinical attachment level (in mm) | 0.00 ± 0.00 | 3.94 ± 0.68 | 4.77 ± 0.64 | < 0.0001 (HS)† |
| 6 months | Gingival index | 0.00 ± 0.00 | 0.49 ± 0.29 | 0.60 ± 0.35 | 0.0009 (S)† |
| | Plaque index | 0.00 ± 0.00 | 0.87 ± 0.42 | 0.24 ± 0.20 | < 0.0001 (HS) † |
| | Probing pocket depth (in mm) | 0.23 ± 0.15[a] | 2.42 ± 0.72[b] | 3.07 ± 0.60[c] | < 0.0001 (HS)* |
| | Clinical attachment level (in mm) | 0.00 ± 0.00 | 2.81 ± 0.78 | 3.75 ± 0.86 | < 0.0001 (HS)† |

HS: Highly Significant; S: Significant

**Table 5: Comparison of BMI and waist circumference across three study groups**

| Characteristics | Level | Group [Mean ± SD] | | | p-value* |
|---|---|---|---|---|---|
| | | Group I (n=30) | Group II (n=30) | Group III (n=30) | |
| BMI ($kg/m^2$) | ≤ 24.9 | 28 (93.33) | 23 (76.67) | 17 (56.67) | |
| | 25-29.9 | 2 (6.67) | 4 (13.33) | 10 (33.33) | |
| | ≥ 30 | 0 | 3 (10) | 3 (10) | |
| | Mean ± SD | 22.00 ± 2.13[a] | 23.63 ± 4.16[a] | 25.67 ± 2.73[b] | 0.0001 (S) |
| Waist Circumference (cm) | Male: < 94 | 9 (30) | 12 (40) | 15 (50) | |
| | ≥ 94 | 1 (3.33) | 2 (6.67) | 2 (6.67) | |
| | Mean ± SD | 81.60 ± 10.17 | 80.57 ± 14.41 | 83.88 ± 4.94 | 0.6601 (NS) |
| | Female < 80 | 10 (33.33) | 10 (33.33) | 7 (23.33) | |
| | ≥ 80 | 10 (33.33) | 6 (20) | 6 (20) | |
| | Mean ± SD | 77.81 ± 12.30 | 78.75 ± 8.37 | 81.85 ± 5.98 | 0.4933 (NS) |

S: Significant; NS: Non-Significant

**Table 6 :Intra group comparison of mean serum and GCF leptin levels**

| Parameter | Group I (n=30) | | | Group II (n=30) | | | Group III (n=30) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pre | Post | P-value* | Pre | Post | P-value* | Pre | Post | p-value* |
| Serum leptin level (ng/ml) | 2.46 ± 0.35[a] | 2.36 ± 0.33[a] | < 0.0001 (HS) | 7.82 ± 0.53[b] | 6.57 ± 0.60[b] | < 0.0001 (HS) | 13.41 ± 0.64[c] | 12.15 ± 1.27[c] | < 0.0001 (HS) |
| GCF leptin level (ng/µl) | 7.28 ± 0.55[a] | 7.98 ± 0.51[a] | < 0.0001 (HS) | 5.19 ± 0.61[b] | 6.34 ± 0.67[b] | < 0.0001 (HS) | 3.97 ± 0.70[c] | 5.00 ± 0.78[c] | < 0.0001 (HS) |

HS: Highly Significant

**Table 7: Inter-group comparison of mean serum and GCF leptin levels**

| Parameter | Pre-treatment | | | p-value‡ | Post-treatment | | | P-value‡ |
|---|---|---|---|---|---|---|---|---|
| | Group I – Healthy | Group II - CP | Group III – CP with T2DM | | Group I - Healthy | Group II - CP | Group III – CP with T2DM | |
| Serum leptin (ng/ml) | 2.46 ± 0.35[a] | 7.82 ±0.53[b] | 13.41 ± 0.64[c] | < 0.0001 (HS) | 2.36 ± 0.33[a] | 6.57 ± 0.60[b] | 12.15 ± 1.27[c] | < 0.0001 (HS) |
| GCF leptin (ng/µl) | 7.28 ± 0.55[a] | 5.19 ± 0.61[b] | 3.97 ± 0.70[c] | < 0.0001 (HS) | 7.98 ± 0.51[a] | 6.34 ± 0.67[b] | 5.00 ± 0.78[c] | < 0.0001 (HS) |

HS: Highly Significant

**Table 8: Intra-group comparison of mean serum and GCF leptin level according to BMI categories**

| BMI (kg/m$^2$) | n | Leptin level [Mean ± SD] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Serum (ng/ml) | | | GCF (ng/µl) | | |
| | | Pre | Post | p-value* | Pre | Post | p-value* |
| ≤ 24.9 (Normal) | 68 | 6.81 ± 4.23 | 6.06 ± 3.80 | < 0.0001 (HS) | 5.97 ± 1.26 | 6.94 ± 1.08 | < 0.0001 (HS) |
| 25-29.9 (Over-weight) | 16 | 11.07 ± 4.06 | 10.17 ± 3.84 | < 0.0001 (HS) | 4.11 ± 1.26 | 5.09 ± 1.18 | < 0.0001 (HS) |
| ≥ 30 (Obese) | 6 | 11.77 ± 3.07 | 9.63 ± 3.31 | 0.1295 (NS) | 3.53 ± 0.73 | 4.43 ± 0.85 | 0.0001 (S) |

HS: Highly Significant; NS: Not Significant

**Table 9: Intra- group comparison of mean HBA1c and Random Blood Sugar level**

| Parameter | Group I | | | Group II | | | Group III | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pre | Post | P-value* | Pre | Post | P-value* | Pre | Post | p-value* |
| HbA1c % | 4.95 ± 0.35[a] | 4.83 ± 0.35[a] | < 0.0001 (HS) | 5.23 ± 0.44[a] | 5.14 ± 0.42[a] | < 0.0001 (HS) | 6.87 ± 0.75[b] | 6.71 ± 0.76[b] | < 0.0001 (HS) |
| RBS (mg/dl) | 98.40 ± 8.45[a] | 96.03 ± 6.90[a] | < 0.0001 (HS) | 107.97 ± 10.51[a] | 103.27 ± 8.63[a] | < 0.0001 (HS) | 178.27 ± 73.69[b] | 156.33 ± 44.07[b] | 0.0038 (S) |

HS: Highly Significant; S: Significant

**Table 10: Inter-group comparison of mean HBA1c and Random Blood Sugar level**

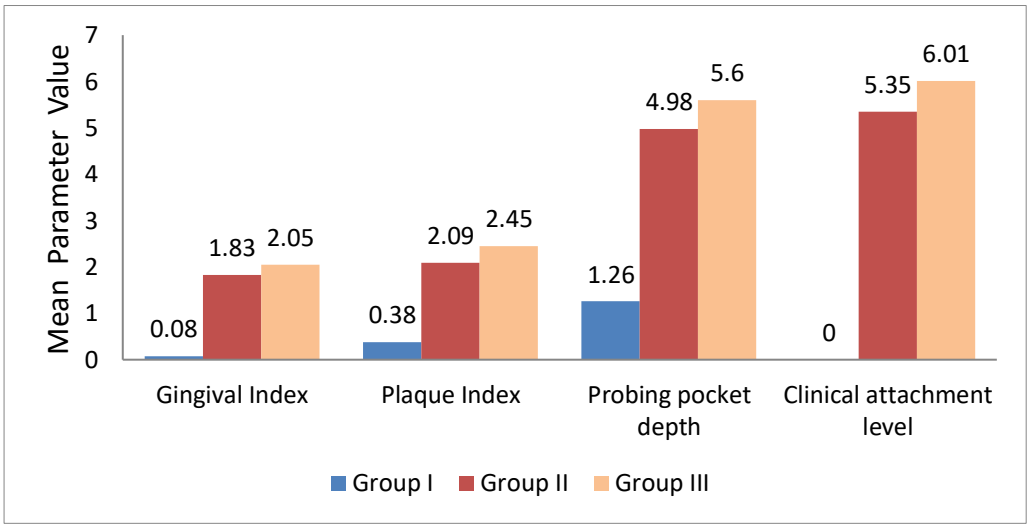
| Parameters | Pre-treatment | | | p-value‡ | Post-treatment | | | p-value‡ |
|---|---|---|---|---|---|---|---|---|
| | Group I – Healthy | Group II - CP | Group III – CP with T2DM | | Group I - Healthy | Group II - CP | Group III – CP with T2DM | |
| HbA1c % | 4.95 ± 0.35[a] | 5.23 ± 0.44[a] | 6.87 ± 0.75[b] | < 0.0001 (HS) | 4.83 ± 0.35[a] | 5.14 ± 0.42[a] | 6.71 ± 0.76[b] | < 0.0001 (HS) |
| RBS (mg/dl) | 98.40 ± 8.45[a] | 107.97 ± 10.51[a] | 178.27 ± 73.69[b] | < 0.0001 (HS) | 96.03 ± 6.90[a] | 103.27 ± 8.63[a] | 156.33 ± 44.07[b] | < 0.0001 (HS) |

HS: Highly Significant; S: Significant

## Graphs

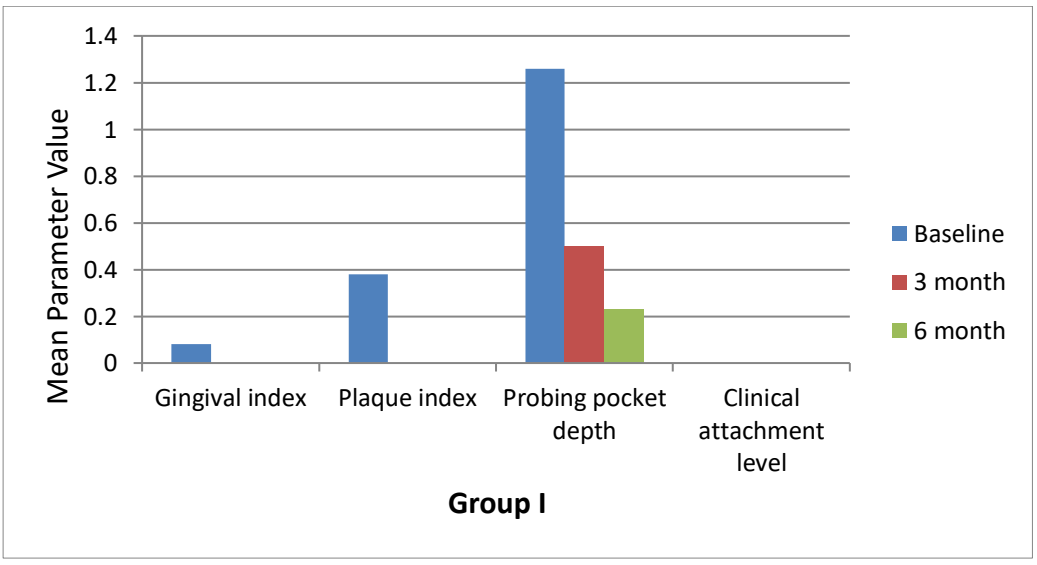
### Graph 1

**Mean values of clinical parameters at baseline in three study groups**



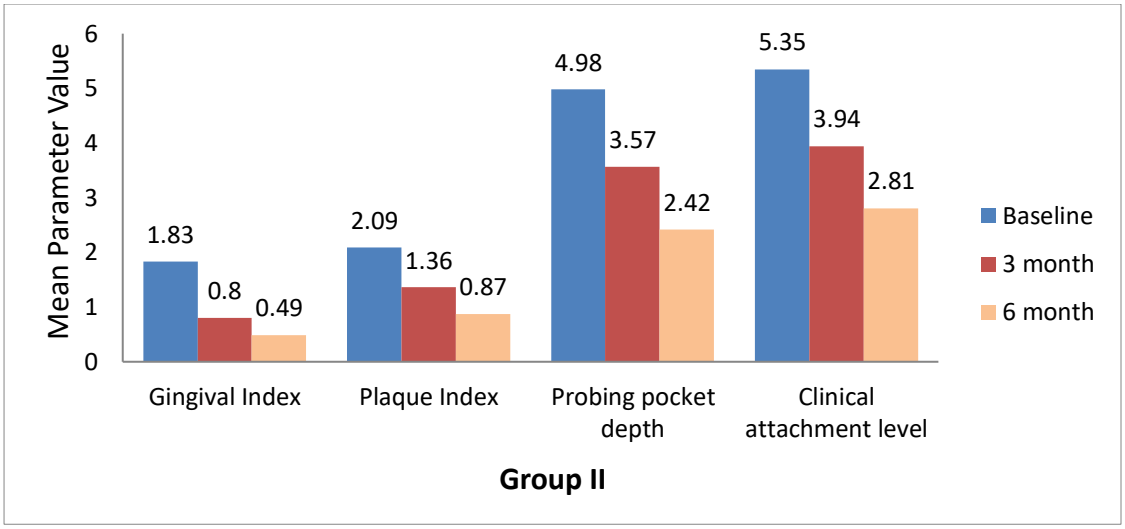

### Graph 2

**Intra-group comparison of clinical parameters across time points in Group I**



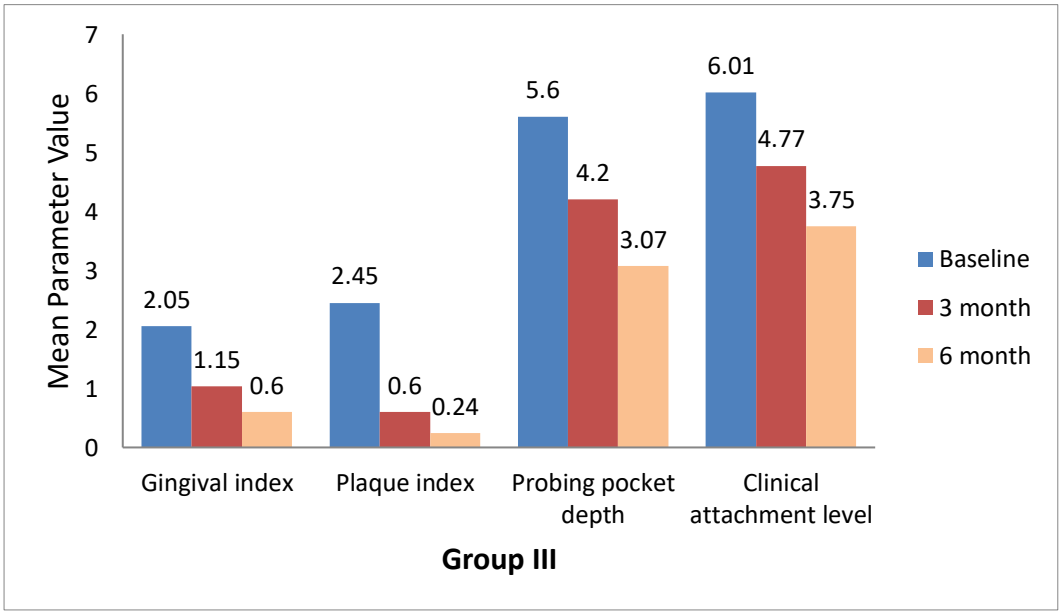
**Graph 3**

**Intra-group comparison of clinical parameters across time points in Group II**



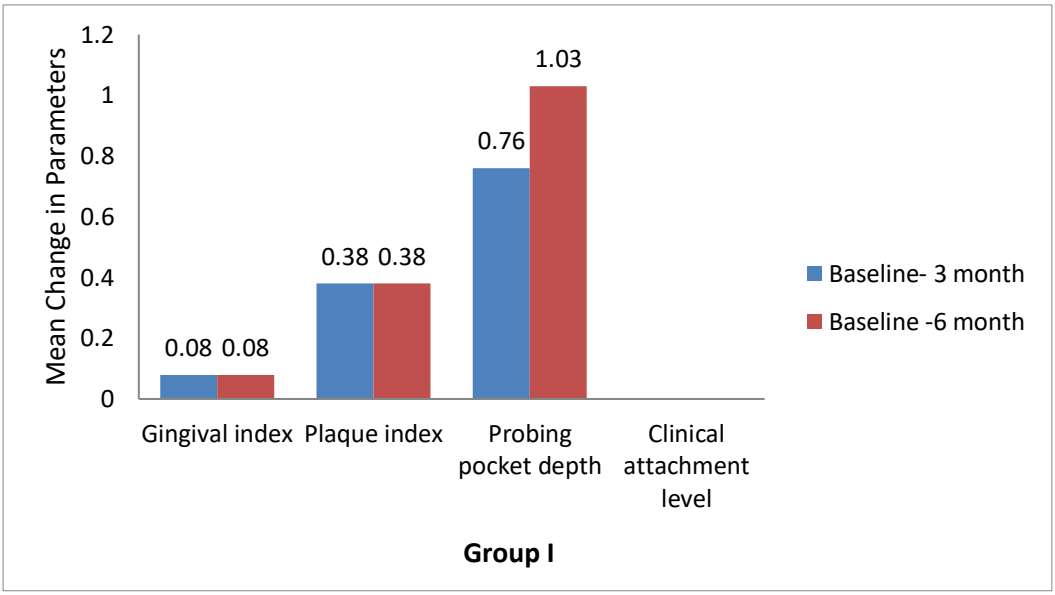

**Graph 4**

**Intra-group comparison of clinical parameters across time points in Group III**



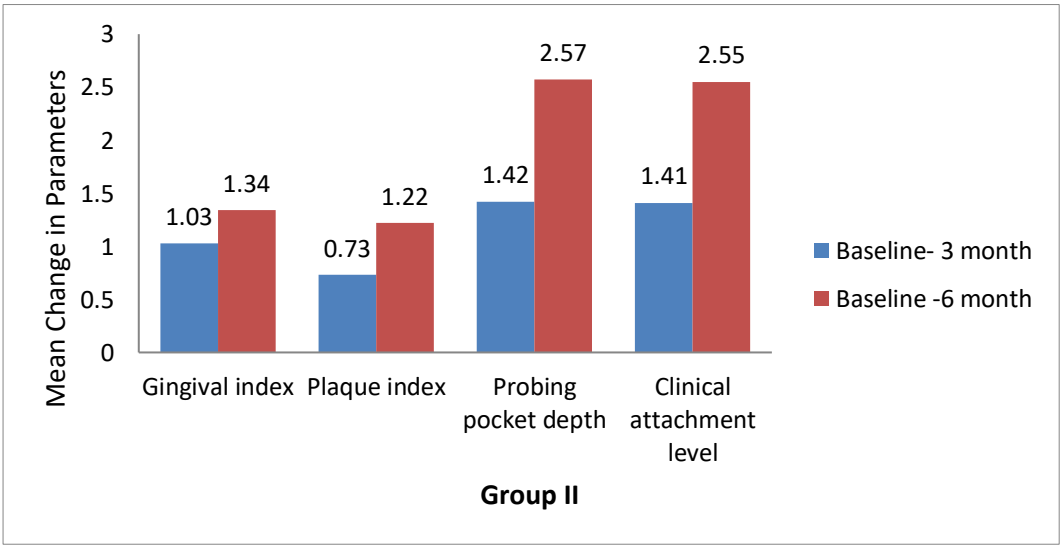
**Graph 5**

**Mean change of clinical parameters at 3 and 6 months with reference to baseline in Group I**



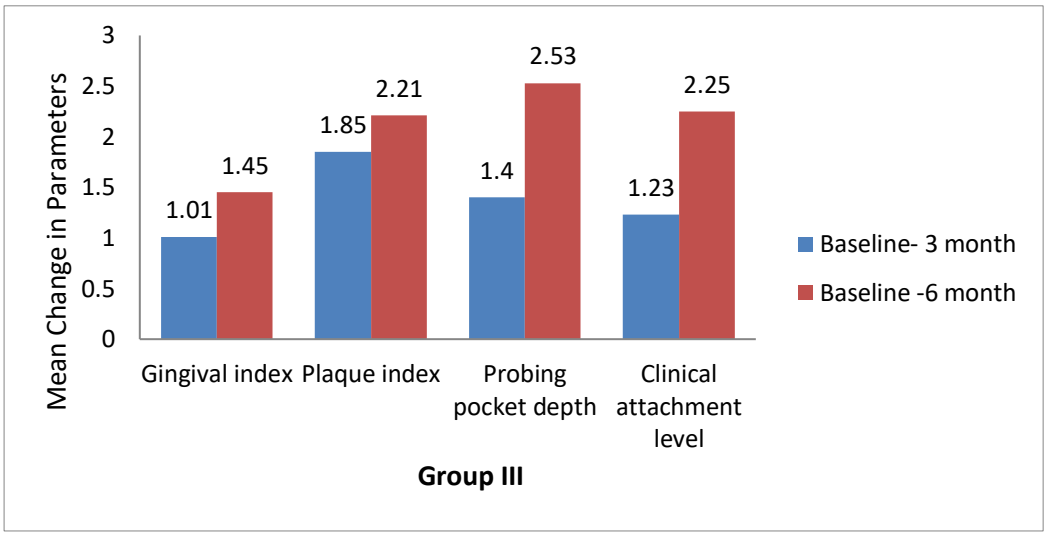

**Graph 6**

**Mean change of clinical parameters at 3 and 6 months with reference to baseline in Group II**



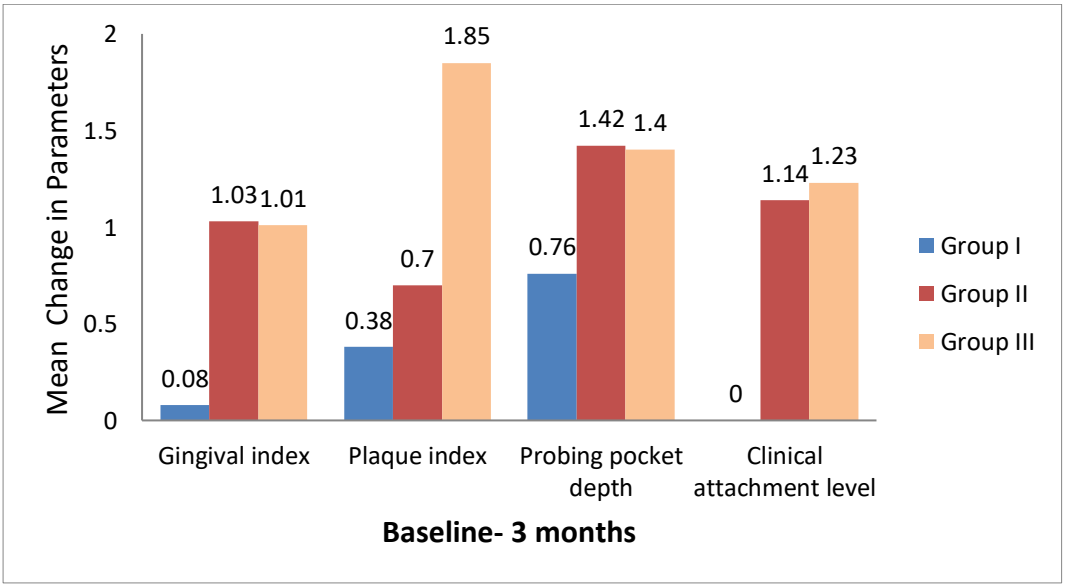
**Graph 7**

**Mean change of clinical parameters at 3 and 6 months with reference to baseline in Group III**



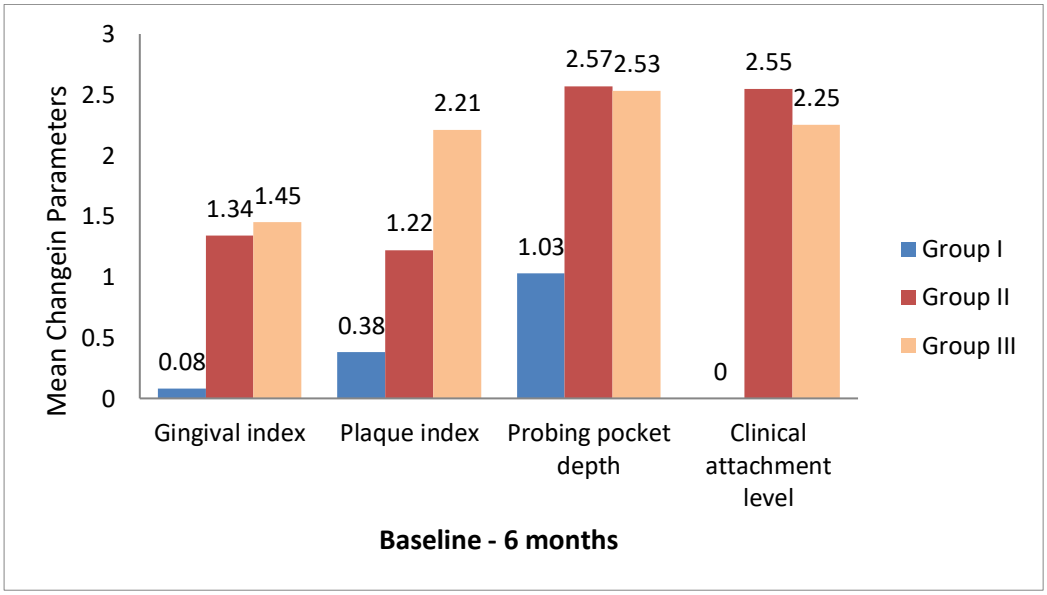

**Graph 8**

**Inter-group comparison of mean change in clinical parameters at 3 months with reference to baseline**



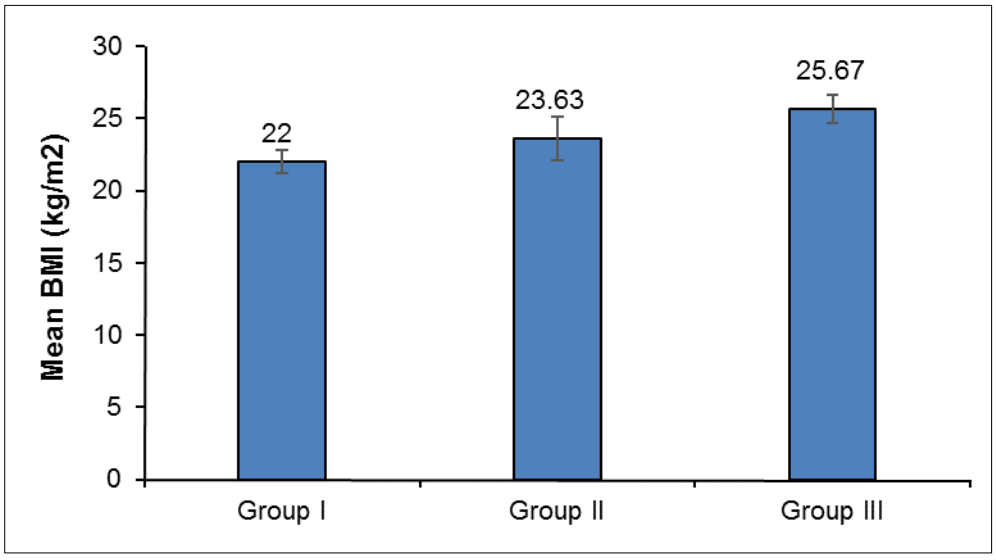
## Graph 9

**Inter-group comparison of mean change in clinical parameters at 6 months with reference to baseline**



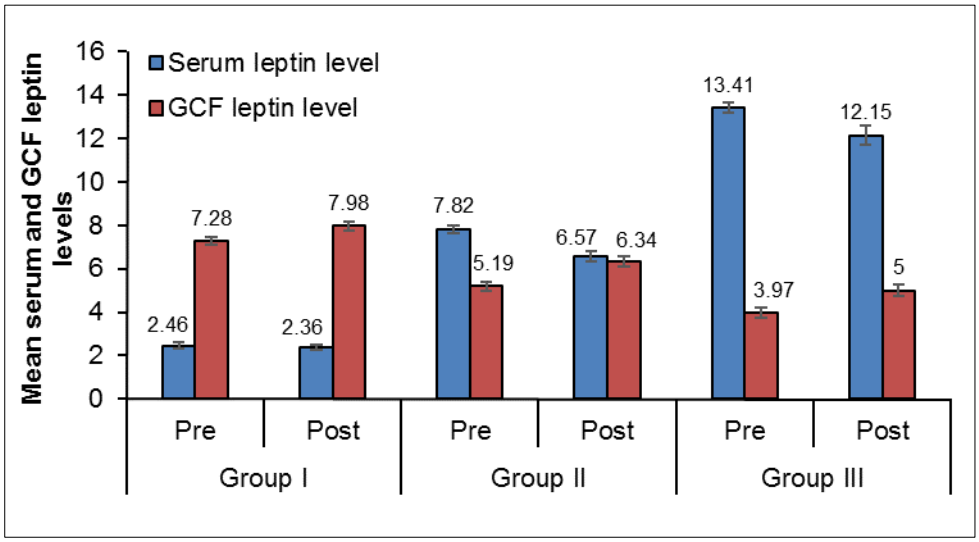

## Graph 10

**Comparison of mean BMI across three study groups**



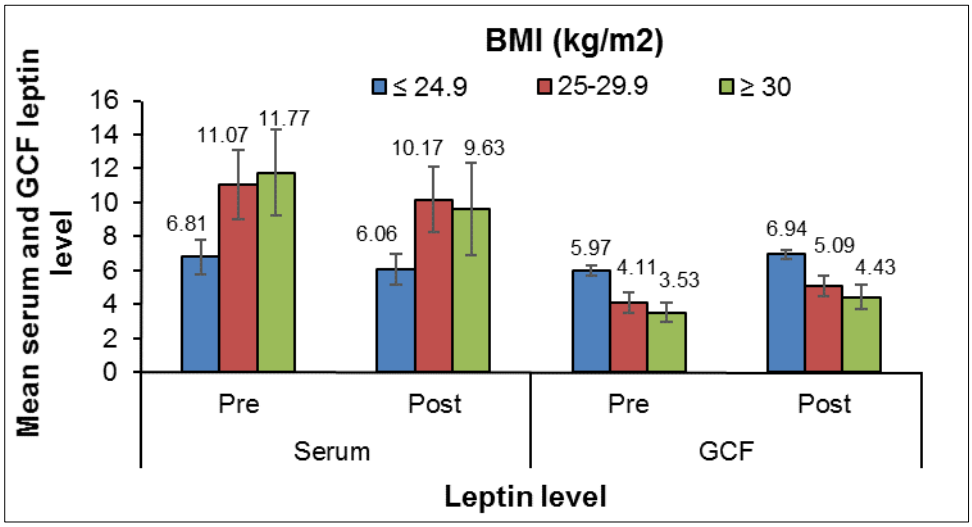
**Graph 11**

**Comparison of pre and post-treatment serum and GCF leptin levels in three study groups**



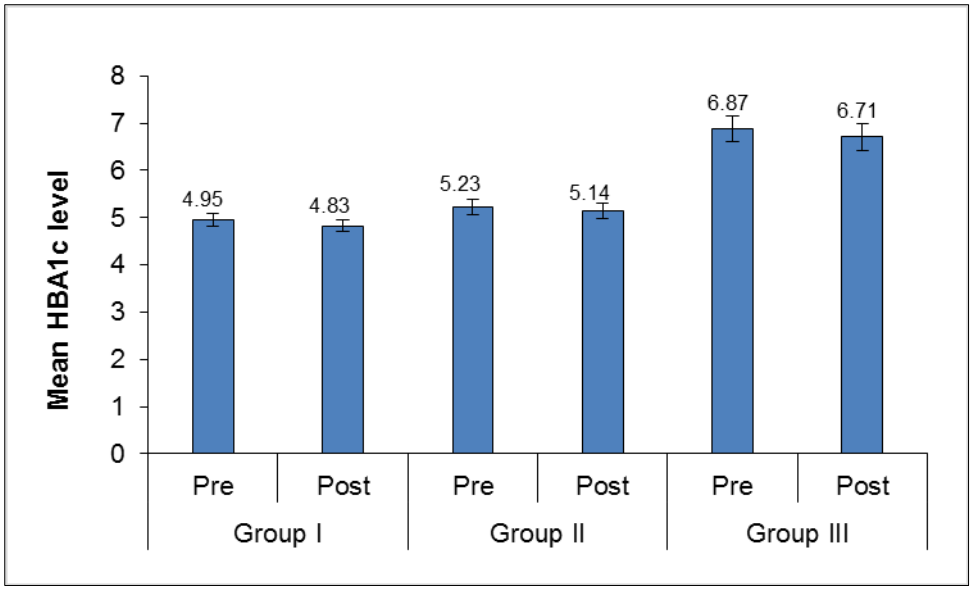

**Graph 12**

**Mean serum and GCF leptin level according to BMI categories**



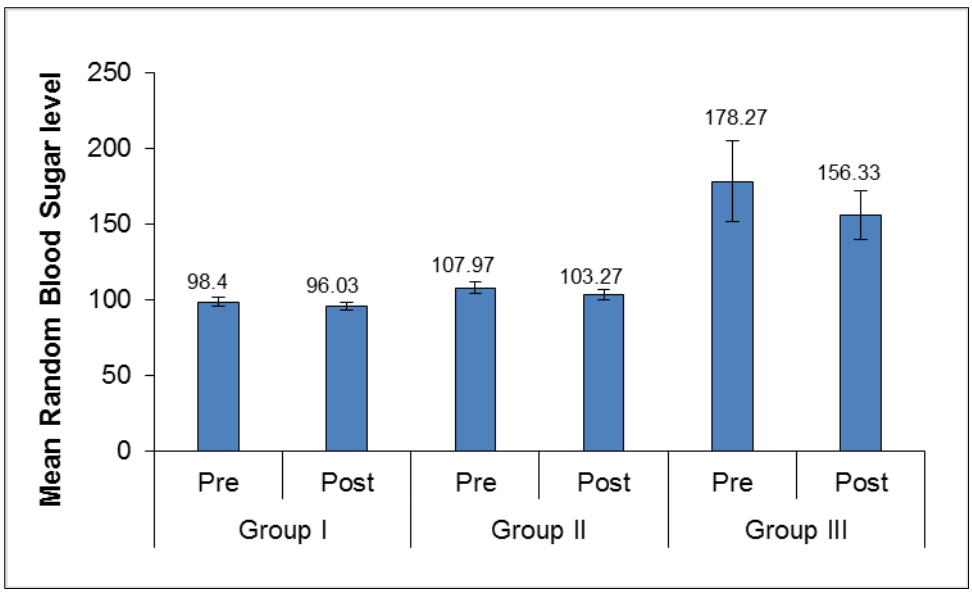
**Graph 13**

**Mean HbA1c levels at pre and post treatment in each group**




**Graph 14**

**Mean RBS levels at pre and post treatment in each group**



# MASTER CHART

## DEMOGRAPHIC CHARACERISTICS IN GROUP I

| Sr. No | Age | Gender | BMI (kg/m$^2$) | Waist Circumference (cm) |
|---|---|---|---|---|
| 1 | 32 | Male | 21.6 | 86 |
| 2 | 50 | Male | 22.8 | 78 |
| 3 | 25 | Male | 21.3 | 93 |
| 4 | 30 | Female | 23.4 | 82 |
| 5 | 23 | Female | 24.2 | 76 |
| 6 | 61 | Male | 21 | 86.5 |
| 7 | 40 | Female | 22.2 | 87.8 |
| 8 | 19 | Female | 24.2 | 55 |
| 9 | 22 | Female | 23.4 | 60 |
| 10 | 25 | Female | 25.1 | 52 |
| 11 | 21 | Male | 21.6 | 98 |
| 12 | 60 | Male | 21 | 76 |
| 13 | 28 | Female | 23.4 | 60 |
| 14 | 23 | Female | 18.5 | 89.3 |
| 15 | 19 | Female | 18.9 | 65 |
| 16 | 19 | Male | 22.9 | 78 |
| 17 | 45 | Male | 24.6 | 68 |
| 18 | 33 | Female | 20.9 | 92 |
| 19 | 23 | Female | 18.9 | 65 |
| 20 | 35 | Male | 26.2 | 81 |
| 21 | 29 | Female | 23.6 | 97 |
| 22 | 24 | Female | 21.5 | 71 |
| 23 | 19 | Female | 18.1 | 81 |
| 24 | 18 | Male | 24.1 | 86 |
| 25 | 22 | Female | 18.9 | 91 |
| 26 | 24 | Female | 20.7 | 75 |
| 27 | 18 | Female | 18.7 | 76 |
| 28 | 23 | Female | 21.8 | 101 |
| 29 | 38 | Female | 22.4 | 71 |
| 30 | 24 | Female | 24.4 | 96 |

## DEMOGRAPHIC CHARACERISTICS IN GROUP II

| Sr. No | Age | Gender | BMI ($kg/m^2$) | Waist Circumference (cm) |
|---|---|---|---|---|
| 1 | 24 | Male | 27.3 | 81.1 |
| 2 | 52 | Male | 23.7 | 89.4 |
| 3 | 30 | Male | 24.5 | 85.5 |
| 4 | 49 | Male | 20.6 | 65 |
| 5 | 35 | Female | 23.5 | 92 |
| 6 | 32 | Male | 25.3 | 78 |
| 7 | 51 | Female | 26.8 | 64.5 |
| 8 | 45 | Female | 22.8 | 60.2 |
| 9 | 45 | Male | 19.5 | 80 |
| 10 | 32 | Male | 24.6 | 80.1 |
| 11 | 47 | Female | 31.4 | 98.4 |
| 12 | 56 | Male | 23.5 | 92.5 |
| 13 | 52 | Male | 23.2 | 79 |
| 14 | 42 | Female | 27.2 | 90 |
| 15 | 42 | Female | 20.8 | 92 |
| 16 | 43 | Female | 24 | 65.5 |
| 17 | 45 | Female | 21.6 | 67.2 |
| 18 | 43 | Female | 20.5 | 85 |
| 19 | 45 | Female | 19.2 | 81.2 |
| 20 | 48 | Female | 23.4 | 80.3 |
| 21 | 51 | Female | 30.8 | 78.9 |
| 22 | 31 | Female | 17.3 | 80.6 |
| 23 | 50 | Male | 23.4 | 73.6 |
| 24 | 39 | Male | 23.9 | 78.6 |
| 25 | 58 | Male | 21.5 | 77.2 |
| 26 | 30 | Female | 23.4 | 81.3 |
| 27 | 60 | Female | 20.4 | 76.2 |
| 28 | 41 | Male | 37 | 79.9 |
| 29 | 34 | Female | 17.4 | 78.8 |
| 30 | 39 | Male | 21.3 | 80.2 |

## DEMOGRAPHIC CHARACERISTICS IN GROUP III

| Sr. No | Age | Gender | BMI (kg/m$^2$) | Waist Circumference (cm) |
|---|---|---|---|---|
| 1 | 48 | Male | 24 | 84.6 |
| 2 | 45 | Male | 25 | 94.2 |
| 3 | 72 | Male | 26.8 | 81.1 |
| 4 | 61 | Female | 30 | 95.1 |
| 5 | 51 | Female | 23 | 79.1 |
| 6 | 48 | Male | 22 | 82.3 |
| 7 | 51 | Female | 23.9 | 81.2 |
| 8 | 39 | Male | 23 | 80.6 |
| 9 | 51 | Male | 27 | 81.5 |
| 10 | 51 | Male | 22.6 | 75.8 |
| 11 | 52 | Female | 23 | 78.6 |
| 12 | 42 | Male | 23.7 | 79.8 |
| 13 | 60 | Male | 26.4 | 81.7 |
| 14 | 51 | Male | 23 | 84.3 |
| 15 | 47 | Male | 23.7 | 87.7 |
| 16 | 51 | Male | 28 | 81.2 |
| 17 | 39 | Female | 29.5 | 76.3 |
| 18 | 49 | Female | 24.4 | 77.9 |
| 19 | 60 | Female | 22.9 | 78.3 |
| 20 | 40 | Female | 23.9 | 74.2 |
| 21 | 60 | Female | 31 | 85.5 |
| 22 | 66 | Male | 29.1 | 87.1 |
| 23 | 63 | Female | 31 | 88.8 |
| 24 | 45 | Female | 20.8 | 87.3 |
| 25 | 62 | Female | 24.7 | 78.2 |
| 26 | 55 | Female | 29 | 84.3 |
| 27 | 58 | Male | 28.4 | 94.2 |
| 28 | 46 | Male | 24 | 88.2 |
| 29 | 52 | Male | 26 | 81.6 |
| 30 | 59 | Male | 24.6 | 78.9 |

## PERIODONTAL CLINICAL PARAMETERS IN GROUP I

| Sr. No | PI | | | GI | | | PPD (mm) | | | CAL (mm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PRE | 3 m | 6 m | PRE | 3 m | 6 m | PRE | 3 m | 6 m | PRE | 3 m | 6m |
| 1 | 0.9 | 0 | 0 | 0.1 | 0 | 0 | 1.22 | 0.52 | 0.1 | 0 | 0 | 0 |
| 2 | 0.8 | 0 | 0 | 0.1 | 0 | 0 | 1.53 | 0.7 | 0.3 | 0 | 0 | 0 |
| 3 | 0.5 | 0 | 0 | 0.1 | 0 | 0 | 1.19 | 0.4 | 0.19 | 0 | 0 | 0 |
| 4 | 0.5 | 0 | 0 | 0 | 0 | 0 | 1.4 | 0.4 | 0.1 | 0 | 0 | 0 |
| 5 | 0.6 | 0 | 0 | 0 | 0 | 0 | 1.05 | 0.45 | 0.1 | 0 | 0 | 0 |
| 6 | 0.08 | 0 | 0 | 0.1 | 0 | 0 | 1.3 | 0.37 | 0.4 | 0 | 0 | 0 |
| 7 | 0.06 | 0 | 0 | 0.1 | 0 | 0 | 1.2 | 0.7 | 0.5 | 0 | 0 | 0 |
| 8 | 0.09 | 0 | 0 | 0.1 | 0 | 0 | 1.41 | 0.5 | 0.33 | 0 | 0 | 0 |
| 9 | 0.08 | 0 | 0 | 0.1 | 0 | 0 | 1.16 | 0.6 | 0.2 | 0 | 0 | 0 |
| 10 | 0.9 | 0 | 0 | 0.1 | 0 | 0 | 1.64 | 0.39 | 0.09 | 0 | 0 | 0 |
| 11 | 0.8 | 0 | 0 | 0.2 | 0 | 0 | 1.15 | 0.5 | 0.4 | 0 | 0 | 0 |
| 12 | 0.5 | 0 | 0 | 0.1 | 0 | 0 | 1.07 | 0.3 | 0.3 | 0 | 0 | 0 |
| 13 | 0.5 | 0 | 0 | 0 | 0 | 0 | 1.7 | 0.5 | 0.4 | 0 | 0 | 0 |
| 14 | 0.6 | 0 | 0 | 0 | 0 | 0 | 1.05 | 0.71 | 0.2 | 0 | 0 | 0 |
| 15 | 0.08 | 0 | 0 | 0.1 | 0 | 0 | 1.2 | 0.4 | 0.16 | 0 | 0 | 0 |
| 16 | 0.06 | 0 | 0 | 0.1 | 0 | 0 | 1.4 | 0.94 | 0.5 | 0 | 0 | 0 |
| 17 | 0.09 | 0 | 0 | 0.1 | 0 | 0 | 1.33 | 1 | 0.4 | 0 | 0 | 0 |
| 18 | 0.08 | 0 | 0 | 0.1 | 0 | 0 | 1.56 | 0.35 | 0.05 | 0 | 0 | 0 |
| 19 | 0.5 | 0 | 0 | 0.1 | 0 | 0 | 1.59 | 0.5 | 0.5 | 0 | 0 | 0 |
| 20 | 0.5 | 0 | 0 | 0 | 0 | 0 | 1.4 | 0.86 | 0.4 | 0 | 0 | 0 |
| 21 | 0.3 | 0 | 0 | 0 | 0 | 0 | 1.18 | 0.7 | 0.1 | 0 | 0 | 0 |
| 22 | 0.3 | 0 | 0 | 0 | 0 | 0 | 1.21 | 0.48 | 0.1 | 0 | 0 | 0 |
| 23 | 0.4 | 0 | 0 | 0.2 | 0 | 0 | 1.07 | 0.51 | 0.3 | 0 | 0 | 0 |
| 24 | 0.1 | 0 | 0 | 0.1 | 0 | 0 | 1.09 | 0.37 | 0.07 | 0 | 0 | 0 |
| 25 | 0.2 | 0 | 0 | 0.1 | 0 | 0 | 1.11 | 0.3 | 0.2 | 0 | 0 | 0 |
| 26 | 0.3 | 0 | 0 | 0.2 | 0 | 0 | 1.18 | 0.4 | 0.18 | 0 | 0 | 0 |
| 27 | 0.7 | 0 | 0 | 0.1 | 0 | 0 | 1.09 | 0.39 | 0.09 | 0 | 0 | 0 |
| 28 | 0.2 | 0 | 0 | 0.1 | 0 | 0 | 1.2 | 0.5 | 0.2 | 0 | 0 | 0 |
| 29 | 0.1 | 0 | 0 | 0.3 | 0 | 0 | 1.12 | 0.2 | 0.1 | 0 | 0 | 0 |
| 30 | 0.6 | 0 | 0 | 0 | 0 | 0 | 1.06 | 0.1 | 0.06 | 0 | 0 | 0 |

## PERIODONTAL CLINICAL PARAMETERS IN GROUP II

| Sr. No | PI | | | GI | | | PPD (mm) | | | CAL (mm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PRE | 3 m | 6 m | PRE | 3 m | 6 m | PRE | 3 m | 6 m | PRE | 3 m | 6m |
| 1 | 2.1 | 1.2 | 0.9 | 2.4 | 1.3 | 1.1 | 5.6 | 4.3 | 3.2 | 6.1 | 4.8 | 3.7 |
| 2 | 2.1 | 1.6 | 1.1 | 2.6 | 1.4 | 1.1 | 4.6 | 3.1 | 1.9 | 4.9 | 3.4 | 2.2 |
| 3 | 2 | 1.1 | 0.6 | 2 | 1 | 0.9 | 4.8 | 3.3 | 2.1 | 5.4 | 3.9 | 2.7 |
| 4 | 2.5 | 1.08 | 0.4 | 1.7 | 0.5 | 0.4 | 4.7 | 3.2 | 2 | 4.9 | 3.4 | 2.2 |
| 5 | 2.5 | 1.5 | 1.3 | 1.5 | 0.5 | 0.2 | 5.7 | 4.7 | 3.8 | 6.3 | 5.3 | 4.4 |
| 6 | 1.4 | 1.1 | 0.4 | 1.8 | 0.9 | 0.4 | 4.5 | 3 | 1.8 | 4.8 | 3.3 | 2.3 |
| 7 | 1.92 | 1.6 | 0.72 | 1.5 | 0.4 | 0.2 | 4.9 | 3.4 | 2.2 | 5.1 | 3.6 | 2.4 |
| 8 | 1.4 | 0.84 | 0.4 | 1.7 | 0.4 | 0.4 | 4.5 | 3 | 1.8 | 4.9 | 3.4 | 2.2 |
| 9 | 1.8 | 1.13 | 0.83 | 1.7 | 0.8 | 0.4 | 4.6 | 3.1 | 1.9 | 5.1 | 3.6 | 2.4 |
| 10 | 1.65 | 1.1 | 0.35 | 2.12 | 1.12 | 0.82 | 4.8 | 3.3 | 2.1 | 5.1 | 3.6 | 2.4 |
| 11 | 2.1 | 1.66 | 1.36 | 2 | 0.9 | 0.7 | 5.1 | 3.7 | 2.6 | 5.4 | 3.9 | 2.7 |
| 12 | 1.4 | 1.65 | 1.1 | 1.5 | 0.5 | 0.2 | 4.9 | 3.4 | 2.2 | 5.1 | 3.7 | 2.6 |
| 13 | 2.2 | 0.92 | 0.62 | 1.8 | 0.7 | 0.2 | 6.1 | 5 | 4.2 | 6.5 | 5.4 | 4.6 |
| 14 | 1.8 | 1.5 | 1.1 | 1.7 | 0.5 | 0.4 | 4.78 | 3.28 | 2.08 | 5.2 | 3.7 | 2.5 |
| 15 | 2.2 | 1.11 | 0.81 | 2.3 | 1.3 | 1 | 5 | 3.5 | 2.3 | 5.4 | 3.9 | 2.7 |
| 16 | 2.4 | 1.06 | 0.5 | 2.1 | 1.2 | 0.8 | 5.2 | 3.9 | 2.8 | 5.5 | 4.2 | 3 |
| 17 | 2.3 | 0.96 | 0.3 | 1.9 | 1.1 | 0.6 | 4.6 | 3.1 | 1.9 | 4.8 | 3.3 | 2.2 |
| 18 | 2.89 | 1.99 | 1.5 | 1.8 | 0.6 | 0.5 | 5.9 | 4.7 | 3.7 | 6.3 | 5 | 4 |
| 19 | 2.85 | 1.84 | 1.6 | 1.5 | 0.4 | 0.2 | 5.6 | 4.2 | 3 | 6.2 | 5 | 4 |
| 20 | 2.1 | 1.58 | 0.28 | 1.5 | 0.3 | 0.1 | 4.8 | 3.3 | 2.1 | 5.2 | 3.8 | 2.6 |
| 21 | 2.1 | 1.1 | 0.8 | 1.6 | 0.7 | 0.3 | 4.9 | 3.4 | 2.2 | 5.2 | 3.7 | 2.5 |
| 22 | 2.2 | 1 | 0.4 | 1.8 | 0.8 | 0.5 | 4.7 | 3.2 | 2 | 5.3 | 3.8 | 2.6 |
| 23 | 2.4 | 1.5 | 1.1 | 1.5 | 0.5 | 0.1 | 4.9 | 3.4 | 2.2 | 5.2 | 3.7 | 2.5 |
| 24 | 1.9 | 1.02 | 0.72 | 2.1 | 1.3 | 0.7 | 4.6 | 3.1 | 1.9 | 4.9 | 3.4 | 2.2 |
| 25 | 2.1 | 1.97 | 1.67 | 1.6 | 0.6 | 0.3 | 5.2 | 3.8 | 2.6 | 5.5 | 4.1 | 2.9 |
| 26 | 2.8 | 1.38 | 1.08 | 2.2 | 1.1 | 0.6 | 6.1 | 5.1 | 4.2 | 6.6 | 5.6 | 4.7 |
| 27 | 2 | 1.5 | 1 | 1.6 | 0.6 | 0.3 | 4.5 | 3 | 1.8 | 4.9 | 3.4 | 2.2 |
| 28 | 2.3 | 1.77 | 1.47 | 2.1 | 1.5 | 0.8 | 5 | 3.6 | 2.4 | 5.2 | 3.7 | 2.5 |
| 29 | 1.9 | 1.6 | 0.43 | 1.9 | 0.5 | 0.4 | 4.5 | 3 | 1.8 | 4.9 | 3.5 | 2.3 |
| 30 | 1.5 | 1.5 | 1.2 | 1.5 | 0.6 | 0.2 | 4.4 | 2.9 | 1.7 | 4.7 | 3.2 | 2 |

## PERIODONTAL CLINICAL PARAMETERS IN GROUP III

| Sr. No | PI | | | GI | | | PPD (mm) | | | CAL (mm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PRE | 3 m | 6 m | PRE | 3 m | 6 m | PRE | 3 m | 6 m | PRE | 3 m | 6m |
| 1 | 2.1 | 0.7 | 0.2 | 2.01 | 1.11 | 0.81 | 5.4 | 3.9 | 2.7 | 5.8 | 4.3 | 3.4 |
| 2 | 2 | 0.7 | 0.3 | 2.5 | 1.3 | 1 | 5.6 | 4.1 | 2.9 | 5.9 | 4.4 | 3.3 |
| 3 | 2.1 | 0.64 | 0.2 | 2.4 | 1.1 | 0.9 | 5.9 | 4.6 | 3.6 | 6.4 | 5.1 | 4.2 |
| 4 | 2.3 | 0.4 | 0.1 | 2.1 | 1.1 | 0.6 | 5.1 | 3.6 | 2.4 | 5.5 | 4 | 3.4 |
| 5 | 2.1 | 0.6 | 0.2 | 1.82 | 1 | 0.3 | 5.2 | 3.7 | 2.5 | 5.7 | 4.2 | 3.3 |
| 6 | 1.9 | 0.7 | 0.4 | 1.68 | 0.5 | 0.2 | 5.7 | 4.3 | 3.2 | 6.1 | 4.7 | 4.1 |
| 7 | 2.1 | 0.5 | 0.2 | 1.23 | 0.33 | 0.03 | 5.2 | 3.7 | 2.5 | 5.6 | 4.1 | 3.6 |
| 8 | 2.4 | 0.7 | 0.5 | 2.31 | 1.1 | 0.8 | 5.4 | 3.9 | 2.7 | 5.8 | 4.3 | 3.5 |
| 9 | 2.5 | 0.3 | 0 | 2.46 | 1.4 | 1 | 5.6 | 4.2 | 3.1 | 6.1 | 4.7 | 3.8 |
| 10 | 2.5 | 0.39 | 0 | 2.31 | 1.41 | 0.84 | 5.2 | 3.7 | 2.5 | 5.7 | 4.2 | 3.5 |
| 11 | 2.2 | 0.6 | 0.1 | 2.7 | 1.8 | 1.2 | 5.4 | 3.9 | 2.7 | 5.9 | 4.4 | 3.4 |
| 12 | 2.8 | 0.4 | 0.2 | 2.5 | 1.3 | 0.5 | 5.8 | 4.5 | 3.4 | 6.2 | 4.9 | 4 |
| 13 | 2.1 | 0.8 | 0.2 | 1.82 | 0.92 | 0.5 | 5.9 | 4.4 | 3.2 | 6.3 | 4.8 | 4.1 |
| 14 | 3 | 0.6 | 0.1 | 2.6 | 1.2 | 0.3 | 6.1 | 4.9 | 3.9 | 6.5 | 5.3 | 4.6 |
| 15 | 2.5 | 0.8 | 0.6 | 1.97 | 1.07 | 0.4 | 5.5 | 4 | 2.8 | 5.9 | 4.4 | 3.3 |
| 16 | 2.4 | 0.6 | 0.4 | 1.4 | 0.4 | 0.1 | 5.7 | 4.3 | 3.2 | 6.2 | 4.8 | 4.1 |
| 17 | 2.6 | 0.9 | 0.5 | 1.94 | 1.04 | 0.74 | 5.4 | 3.9 | 2.7 | 5.8 | 4.3 | 3.5 |
| 18 | 3 | 1 | 0.7 | 1.72 | 0.5 | 0.2 | 6.2 | 5.1 | 4.2 | 6.6 | 5.5 | 4.8 |
| 19 | 2.4 | 0.35 | 0.05 | 2.22 | 1.32 | 0.9 | 5.7 | 4.3 | 3.2 | 6.1 | 4.7 | 4.1 |
| 20 | 2.5 | 0.32 | 0 | 1.95 | 1.05 | 0.34 | 5.6 | 4.1 | 2.9 | 6 | 4.5 | 3.9 |
| 21 | 2.7 | 0.62 | 0.2 | 1.81 | 0.91 | 0.61 | 5.7 | 4.4 | 3.3 | 6.2 | 4.7 | 3.8 |
| 22 | 2.8 | 0.5 | 0 | 1.67 | 0.77 | 0.4 | 6.1 | 5 | 4.1 | 6.5 | 5.2 | 4.7 |
| 23 | 2.2 | 0.45 | 0.15 | 1.91 | 1.01 | 0.38 | 5.4 | 3.9 | 2.7 | 5.8 | 4.3 | 3.6 |
| 24 | 2.3 | 0.52 | 0.22 | 2.13 | 1.23 | 0.93 | 5.2 | 3.7 | 2.5 | 5.6 | 4.1 | 3.5 |
| 25 | 2.3 | 0.7 | 0.4 | 2.57 | 1.67 | 1.3 | 5.1 | 3.6 | 2.4 | 5.4 | 3.9 | 3 |
| 26 | 3 | 0.6 | 0.3 | 2.74 | 1.7 | 1.1 | 6.5 | 5.4 | 4.5 | 6.8 | 5.7 | 4.6 |
| 27 | 3 | 0.5 | 0 | 2.14 | 1.24 | 0.94 | 6.3 | 5.1 | 4.1 | 6.6 | 5.1 | 4.5 |
| 28 | 2.3 | 0.9 | 0.5 | 1.78 | 0.88 | 0.3 | 5.1 | 3.6 | 2.4 | 5.5 | 4 | 3.2 |
| 29 | 2.6 | 1 | 0.5 | 1.37 | 0.25 | 0 | 5.2 | 3.7 | 2.5 | 5.5 | 4 | 3.2 |
| 30 | 2.9 | 0.4 | 0 | 1.75 | 0.85 | 0.55 | 5.8 | 4.4 | 3.3 | 6.2 | 4.8 | 4 |

## SERUM AND GCF LEPTIN CONCENTRATION IN GROUP I

| Sr. No | Serum Leptin (ng/ml) | | GCF Leptin(ng/µl) | |
|---|---|---|---|---|
| | **PRE** | **POST** | **PRE** | **POST** |
| 1 | 2.2 | 2.1 | 6.8 | 7.5 |
| 2 | 1.8 | 1.8 | 6.5 | 7.2 |
| 3 | 2.2 | 2.1 | 6.9 | 7.6 |
| 4 | 2.4 | 2.3 | 7.5 | 8.2 |
| 5 | 2.6 | 2.5 | 7.9 | 8.6 |
| 6 | 2.8 | 2.6 | 7.1 | 7.8 |
| 7 | 2.9 | 2.8 | 8.1 | 8.8 |
| 8 | 2.7 | 2.6 | 6.9 | 7.6 |
| 9 | 2.9 | 2.8 | 7.4 | 8.1 |
| 10 | 2.4 | 2.4 | 7.6 | 8.3 |
| 11 | 1.7 | 1.7 | 7.6 | 8.3 |
| 12 | 2.3 | 2.1 | 7.4 | 8.1 |
| 13 | 2.9 | 2.8 | 8.2 | 8.9 |
| 14 | 2.5 | 2.4 | 7.2 | 7.9 |
| 15 | 1.9 | 1.9 | 6.5 | 7.2 |
| 16 | 2.3 | 2.2 | 6.5 | 7.2 |
| 17 | 3 | 2.7 | 7.6 | 8.3 |
| 18 | 2.7 | 2.7 | 7.1 | 7.8 |
| 19 | 2.8 | 2.7 | 7.4 | 8.1 |
| 20 | 2.9 | 2.8 | 6.2 | 6.9 |
| 21 | 2.2 | 2.1 | 7.6 | 8.3 |
| 22 | 2.6 | 2.5 | 7.3 | 8 |
| 23 | 2.4 | 2.3 | 6.4 | 7.1 |
| 24 | 2.1 | 2 | 6.9 | 7.6 |
| 25 | 2.3 | 2 | 7.8 | 8.5 |
| 26 | 2 | 1.9 | 6.9 | 7.6 |
| 27 | 2.6 | 2.5 | 7.5 | 8.2 |
| 28 | 2.5 | 2.5 | 7.6 | 8.3 |
| 29 | 2.4 | 2.3 | 8.2 | 8.9 |
| 30 | 2.9 | 2.8 | 7.9 | 8.6 |

## SERUM AND GCF LEPTIN CONCENTRATION IN GROUP II

| Sr. No | Serum Leptin (ng/ml) | | GCF Leptin(ng/µl) | |
|---|---|---|---|---|
| | **PRE** | **POST** | **PRE** | **POST** |
| 1 | 8.6 | 7.4 | 4.5 | 5.6 |
| 2 | 7.6 | 6.3 | 5.6 | 6.8 |
| 3 | 7.8 | 6.5 | 4.9 | 6.1 |
| 4 | 7.3 | 6 | 5.7 | 6.9 |
| 5 | 7.9 | 6.6 | 5.3 | 6.5 |
| 6 | 8.1 | 7.1 | 4.3 | 5.3 |
| 7 | 8.3 | 7.2 | 4.7 | 5.8 |
| 8 | 7.4 | 6.1 | 5.8 | 7 |
| 9 | 7.6 | 6.3 | 5.7 | 6.9 |
| 10 | 7.9 | 6.6 | 4.7 | 5.9 |
| 11 | 9.1 | 8.1 | 4.2 | 5.2 |
| 12 | 7.6 | 6.3 | 5.5 | 6.7 |
| 13 | 7.5 | 6.2 | 5.7 | 6.9 |
| 14 | 8.6 | 7.4 | 4.4 | 5.4 |
| 15 | 7.8 | 6.5 | 5.9 | 7.1 |
| 16 | 7.7 | 6.4 | 5.1 | 6.3 |
| 17 | 7.2 | 5.9 | 5.8 | 7 |
| 18 | 7.9 | 6.6 | 5.8 | 7 |
| 19 | 7.5 | 6.2 | 5.9 | 7.1 |
| 20 | 7.4 | 6.2 | 5.4 | 6.6 |
| 21 | 8.9 | 7.6 | 4.3 | 5.3 |
| 22 | 7.4 | 6.1 | 4.1 | 5.3 |
| 23 | 7.5 | 6.2 | 5.6 | 6.8 |
| 24 | 7.7 | 6.5 | 5.1 | 6.2 |
| 25 | 7.2 | 5.9 | 5.5 | 6.7 |
| 26 | 7.6 | 6.3 | 5.1 | 6.3 |
| 27 | 7.5 | 6.2 | 5.3 | 6.5 |
| 28 | 8.9 | 7.9 | 4.1 | 5.1 |
| 29 | 7.1 | 5.9 | 5.8 | 7 |
| 30 | 7.9 | 6.6 | 5.8 | 7 |

## SERUM AND GCF LEPTIN CONCENTRATION IN GROUP III

| Sr. No | Serum Leptin (ng/ml) | | GCF Leptin(ng/µl) | |
|---|---|---|---|---|
| | **PRE** | **POST** | **PRE** | **POST** |
| 1 | 12.6 | 11.5 | 4.3 | 5.4 |
| 2 | 12.9 | 11.8 | 3.9 | 4.9 |
| 3 | 13.7 | 12.8 | 3.7 | 4.7 |
| 4 | 14.5 | 13.7 | 2.9 | 3.9 |
| 5 | 12.7 | 11.6 | 4.2 | 5.2 |
| 6 | 12.7 | 11.6 | 4.8 | 5.9 |
| 7 | 12.9 | 11.8 | 4.5 | 5.6 |
| 8 | 12.9 | 11.8 | 4.6 | 5.7 |
| 9 | 13.8 | 13 | 3.5 | 4.4 |
| 10 | 12.8 | 11.7 | 4.5 | 5.6 |
| 11 | 13.1 | 12 | 4.3 | 5.4 |
| 12 | 13.1 | 12 | 4.2 | 5.3 |
| 13 | 13.8 | 12.8 | 3.6 | 4.6 |
| 14 | 12.5 | 11.4 | 4.8 | 5.9 |
| 15 | 13.3 | 12.2 | 4.5 | 5.6 |
| 16 | 14.1 | 13.1 | 3.1 | 4.1 |
| 17 | 14.2 | 13.3 | 2.8 | 3.6 |
| 18 | 13.4 | 12.3 | 4.1 | 5.2 |
| 19 | 12.8 | 11.7 | 4.6 | 5.7 |
| 20 | 12.9 | 11.8 | 4.5 | 5.6 |
| 21 | 14.7 | 14 | 2.8 | 3.7 |
| 22 | 13.9 | 12.8 | 3.1 | 4.2 |
| 23 | 14.5 | 6.5 | 2.9 | 3.7 |
| 24 | 12.9 | 11.8 | 4.9 | 6 |
| 25 | 13.6 | 12.5 | 4.4 | 5.3 |
| 26 | 14.3 | 13.3 | 2.9 | 4 |
| 27 | 13.9 | 12.9 | 3.3 | 4.5 |
| 28 | 13.1 | 12 | 4.7 | 5.8 |
| 29 | 13.6 | 12.6 | 4.1 | 5.2 |
| 30 | 13.2 | 12.1 | 4.5 | 5.5 |

## HbA1c AND RBS LEVELS IN GROUP I

| Sr. No | HbA1c (%) | | RBS (mg/dl) | |
|---|---|---|---|---|
| | **PRE** | **POST** | **PRE** | **POST** |
| 1 | 4.8 | 4.7 | 88 | 89 |
| 2 | 4.9 | 4.9 | 96 | 95 |
| 3 | 5.1 | 5 | 98 | 96 |
| 4 | 5.3 | 5 | 92 | 90 |
| 5 | 4.6 | 4.5 | 91 | 91 |
| 6 | 4.2 | 4.2 | 104 | 101 |
| 7 | 5.3 | 5.1 | 97 | 96 |
| 8 | 5 | 5 | 92 | 90 |
| 9 | 4.7 | 4.5 | 98 | 97 |
| 10 | 5.3 | 5.2 | 101 | 99 |
| 11 | 4.2 | 4.2 | 98 | 96 |
| 12 | 4.4 | 4.3 | 101 | 99 |
| 13 | 4.8 | 4.5 | 95 | 92 |
| 14 | 5.2 | 5.1 | 89 | 89 |
| 15 | 5.1 | 5.1 | 92 | 88 |
| 16 | 4.5 | 4.4 | 111 | 104 |
| 17 | 5 | 5 | 114 | 110 |
| 18 | 5.5 | 5.3 | 90 | 90 |
| 19 | 5 | 5 | 113 | 108 |
| 20 | 5.1 | 5 | 104 | 102 |
| 21 | 5.3 | 5.2 | 98 | 96 |
| 22 | 4.6 | 4.4 | 82 | 82 |
| 23 | 5 | 4.8 | 106 | 102 |
| 24 | 5.3 | 5.1 | 95 | 95 |
| 25 | 4.6 | 4.2 | 110 | 101 |
| 26 | 5 | 5 | 87 | 86 |
| 27 | 5.5 | 5.3 | 91 | 89 |
| 28 | 5 | 4.8 | 104 | 99 |
| 29 | 5 | 4.9 | 113 | 108 |
| 30 | 5.2 | 5.1 | 102 | 101 |

## HbA1c AND RBS LEVELS IN GROUP II

| Sr. No | HbA1c (%) | | RBS (mg/dl) | |
|---|---|---|---|---|
| | **PRE** | **POST** | **PRE** | **POST** |
| 1 | 5.6 | 5.5 | 101 | 99 |
| 2 | 5.2 | 5.1 | 112 | 103 |
| 3 | 5.1 | 5 | 104 | 98 |
| 4 | 4.9 | 4.9 | 102 | 101 |
| 5 | 4.9 | 4.9 | 99 | 95 |
| 6 | 5.7 | 5.5 | 108 | 107 |
| 7 | 5.5 | 5.4 | 136 | 120 |
| 8 | 4.7 | 4.7 | 99 | 92 |
| 9 | 4.9 | 4.8 | 116 | 114 |
| 10 | 5.6 | 5.5 | 110 | 106 |
| 11 | 6 | 5.9 | 123 | 111 |
| 12 | 5.1 | 5 | 103 | 101 |
| 13 | 5.3 | 5.2 | 94 | 92 |
| 14 | 5.8 | 5.7 | 112 | 109 |
| 15 | 4.3 | 4.1 | 103 | 101 |
| 16 | 5.3 | 5.2 | 119 | 105 |
| 17 | 5.3 | 5.2 | 115 | 110 |
| 18 | 5 | 5 | 95 | 92 |
| 19 | 4.8 | 4.7 | 101 | 96 |
| 20 | 5.3 | 5.1 | 104 | 99 |
| 21 | 5.9 | 5.7 | 120 | 114 |
| 22 | 4.4 | 4.4 | 98 | 97 |
| 23 | 5 | 5 | 115 | 113 |
| 24 | 5.4 | 5.3 | 95 | 93 |
| 25 | 4.9 | 4.8 | 102 | 101 |
| 26 | 5.5 | 5.4 | 97 | 92 |
| 27 | 5.1 | 5 | 114 | 110 |
| 28 | 6.1 | 6 | 130 | 125 |
| 29 | 5.4 | 5.3 | 110 | 103 |
| 30 | 5 | 4.9 | 102 | 99 |

## HbA1c AND RBS LEVELS IN GROUP III

| Sr. No | HbA1c (%) | | RBS (mg/dl) | |
|---|---|---|---|---|
| | **PRE** | **POST** | **PRE** | **POST** |
| 1 | 7.9 | 7.8 | 480 | 266 |
| 2 | 7.7 | 7.6 | 235 | 210 |
| 3 | 6.9 | 6.7 | 93 | 93 |
| 4 | 7.9 | 7.8 | 277 | 258 |
| 5 | 6.2 | 6.1 | 93 | 90 |
| 6 | 7.8 | 7.7 | 187 | 143 |
| 7 | 6.7 | 6.6 | 102 | 96 |
| 8 | 7.8 | 7.6 | 180 | 169 |
| 9 | 6.9 | 6.8 | 281 | 249 |
| 10 | 5.5 | 5.3 | 143 | 136 |
| 11 | 6.7 | 6.5 | 172 | 153 |
| 12 | 7.2 | 6.9 | 163 | 154 |
| 13 | 7 | 6.7 | 148 | 136 |
| 14 | 5.8 | 5.6 | 108 | 104 |
| 15 | 7 | 6.8 | 189 | 165 |
| 16 | 6.9 | 6.8 | 161 | 149 |
| 17 | 6.7 | 6.2 | 148 | 135 |
| 18 | 7.2 | 7.1 | 159 | 151 |
| 19 | 8 | 7.9 | 178 | 163 |
| 20 | 6.6 | 6.4 | 123 | 117 |
| 21 | 7.8 | 7.6 | 187 | 174 |
| 22 | 7.5 | 7.4 | 258 | 205 |
| 23 | 6.9 | 6.8 | 159 | 144 |
| 24 | 5.3 | 5.3 | 130 | 123 |
| 25 | 6.4 | 6.3 | 167 | 159 |
| 26 | 6.9 | 6.8 | 156 | 152 |
| 27 | 6.5 | 6.4 | 168 | 145 |
| 28 | 6.8 | 6.7 | 175 | 144 |
| 29 | 5.9 | 5.7 | 159 | 149 |
| 30 | 5.7 | 5.5 | 169 | 158 |

**Effect of Non Surgical Periodontal Therapy on GCF and Serum Leptin Levels in Periodontally Healthy, Chronic Periodontitis and Chronic Periodontitis with Type 2 Diabetes Mellitus Patients**

## CASE HISTORY PROFORMA

**NAME:** **OPD NO:**

**AGE/SEX:** **DATE:**

**ADDRESS:** **PHONE NO:**

**OCCUPATION:**

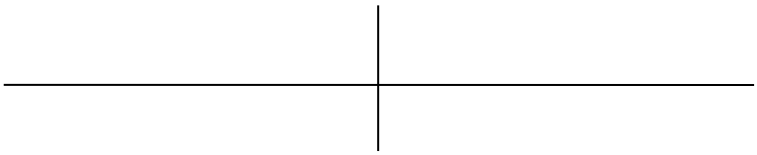
**CHIEF COMPLAINT:**

**PAST DENTAL HISTORY:**

**PAST MEDICAL HISTORY:**

**ORAL HYGIENE HABIT:**

**TEETH PRESENT:**



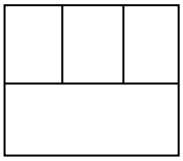
**Physical Examination:**

1. Height =
2. Weight =
3. Waist circumference =
4. Body mass index = $\frac{\text{weight(kg)}}{\text{Height (m}^2\text{)}}$ =

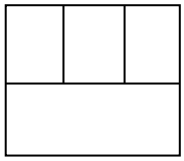
**Biochemical analysis**

1. RBS levels (Baseline) =
2. RBS levels (6 months)=
3. HbA1c (Baseline)=
4. HbA1c (6 months)

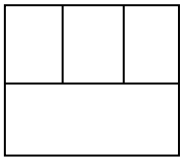
**GINGIVAL INDEX *(Loe & Sillness 1963*) Baseline**



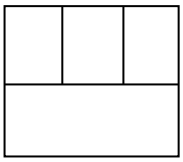
16



12

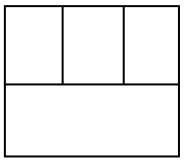


24



44

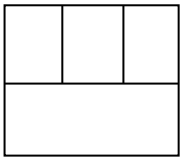
32



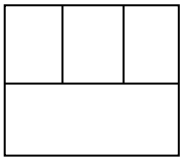
36

**Score:** $\frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$ **SCORE:**

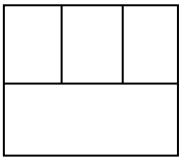
**GINGIVAL INDEX (After 3 months)**



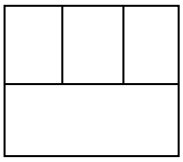
16



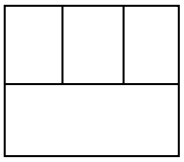
12



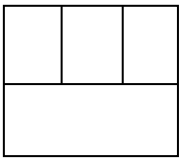
24



44



32

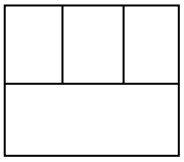


36

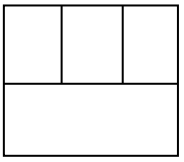
**Score:** $\frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$ **SCORE:**

**GINGIVAL INDEX (After 6 months)**

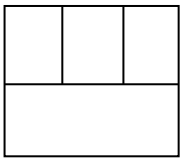
16



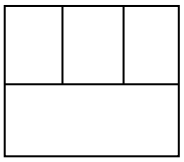
12



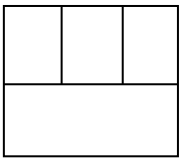
24



44



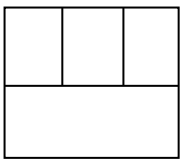
32



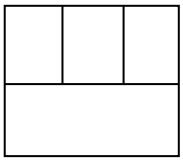
36

**Score:** $\frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$ **SCORE:**

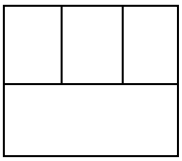
**PLAQUE INDEX *(Sillness and Loe 1964)* Baseline**



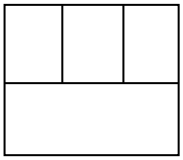
16



12

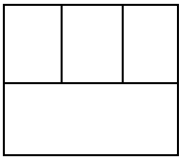


24



44

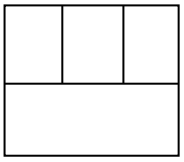
32



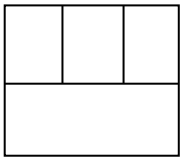
36

**Score:** $\frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$ **SCORE:**

**PLAQUE INDEX (After 3 months)**

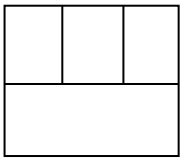


16

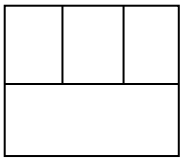


12

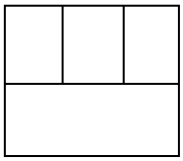
24



44



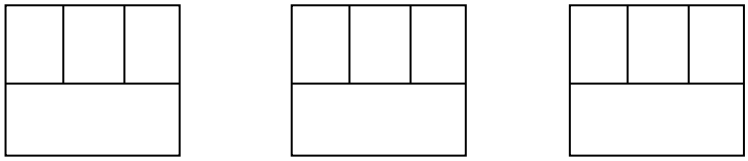
32



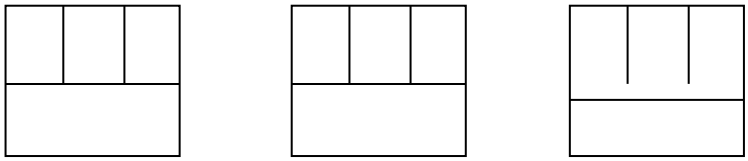
36

**Score:** $\frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$ **SCORE:**

**PLAQUE INDEX (After 6months)**



16 12 24

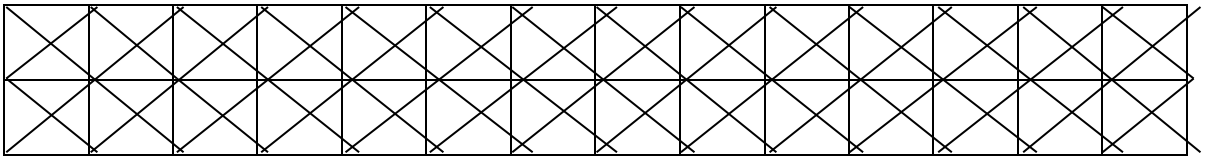


44 32 36

**Score:** $\frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$ **SCORE:**

**PROBING POCKET DEPTH (mm): Baseline**

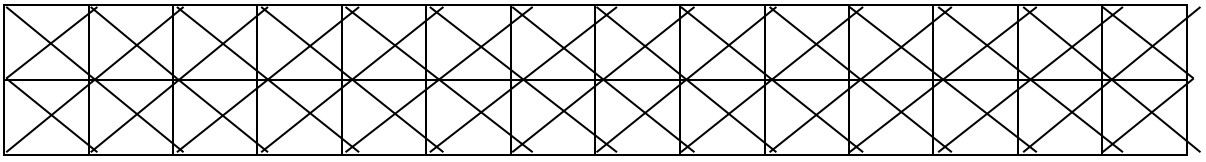
17 16 15 14 13 12 11 21 22 23 24 25 26 27



47 46 45 44 43 42 41 31 32 33 34 35 36 37

**PROBING POCKET DEPTH (mm): After 3 months**

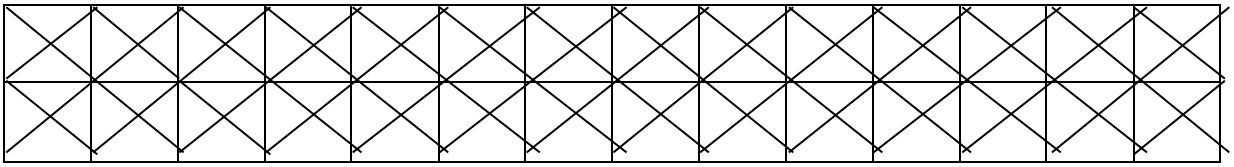
17 16 15 14 13 12 11 21 22 23 24 25 26 27



47 46 45 44 43 42 41 31 32 33 34 35 36 37

**PROBING POCKET DEPTH (mm): After 6 months**

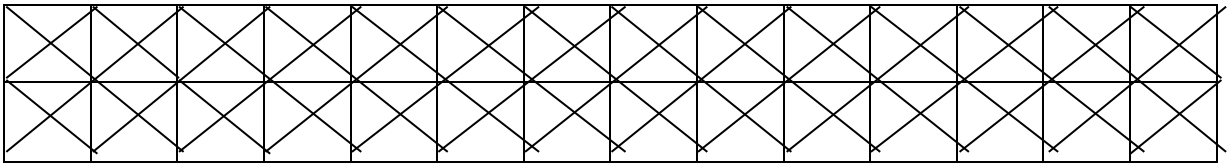
17 16 15 14 13 12 11 21 22 23 24 25 26 27



47 46 45 44 43 42 41 31 32 33 34 35 36 37

**CLINICAL ATTACHMENT LEVELS (mm): Baseline**

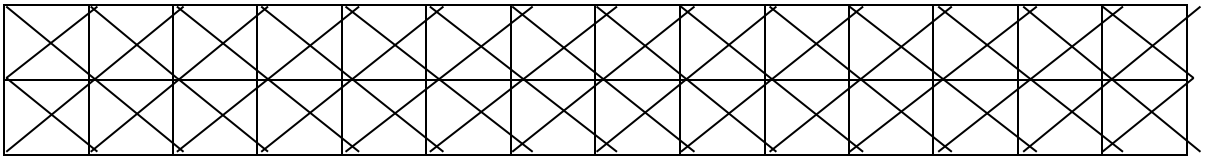
17 16 15 14 13 12 11 21 22 23 24 25 26 27



47 46 45 44 43 42 41 31 32 33 34 35 36 37

**CLINICAL ATTACHMENT LEVELS (mm): After 3 months**

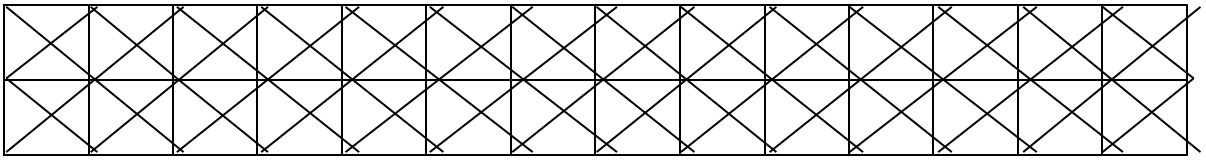
17 16 15 14 13 12 11 21 22 23 24 25 26 27



47 46 45 44 43 42 41 31 32 33 34 35 36 37

**CLINICAL ATTACHMENT LEVELS (mm): After 6 months**

17 16 15 14 13 12 11 21 22 23 24 25 26 27



47 46 45 44 43 42 41 31 32 33 34 35 36 37

**Clinical Diagnosis:**

| **Parameters** | **Group I Healthy** | **Group II CP** | **Group III CP+T2DM** | **Group II CP** | **Group III CP+T2DM** |
|---|---|---|---|---|---|
| | | **Baseline** | | **After 6 months** | |
| **Serum leptin conc.(ng/ml)** | | | | | |
| **GCF leptin conc.(ng/µl)** | | | | | |

## (Confidential)

## Informed Consent Form

**"**Effect of Non Surgical Periodontal Therapy on GCF and Serum Leptin Levels in Periodontally Healthy, Chronic Periodontitis and Chronic Periodontitis with Type 2 Diabetes Mellitus Patients"

Mr./Master/Mrs./Miss._____

Resident of: _____

_____aged _____ years, excercising my free will/choice, without any pressure/lure of incentive in any form, hereby give my consent for the project to be conducted.

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.

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