EVALUATION OF SERUM AND SALIVARY LEPTIN CONCENTRATIONS IN PERIODONTALLY HEALTHY AND CHRONIC PERIODONTITIS INDIVIDUALS BEFORE AND AFTER NON SURGICAL PERIODONTAL THERAPY

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THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH II

PERIODONTICS

2015- 2018

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation titled **"EVALUATION OF SERUM AND SALIVARY LEPTIN CONCENTRATIONS IN PERIODONTALLY HEALTHY AND CHRONIC PERIODONTITIS INDIVIDUALS BEFORE AND AFTER NON SURGICAL PERIODONTAL THERAPY"** is a bonafide work done **Dr.P.S.VIOLA ESTHER,** Postgraduate student, during the course of the study for the degree of **MASTER OF DENTAL SURGERY** in the specialty of **DEPARTMENT OF PERIODONTICS,** Vivekanandha Dental College for Women, Tiruchengode, during the period of 2015-2018.

Date: 18/1/2018
Place: Tiruchengode

Dr. S.ELANCHEZHIYAN, M.D.S.
Professor and Head
Department of Periodontics,
Vivekanandha Dental College for Women
Tiruchengode - 637205, Tamilnadu, India

Signature of H.O.D & Guide

Dr.S.ELANCHEZHIYAN M.D.S,
Professor and Head,
Department of Periodontics,
Vivekanandha Dental College for Women

ENDORSEMENT BY THE HEAD OF THE DEPARTMENT AND HEAD OF THE INSTITUTION

This is to certify that **Dr.P.S.VIOLA ESTHER,** Post Graduate student (2015-2018) in the **DEPARTMENT OF PERIODONTICS,** Vivekanandha Dental College for Women, has done this dissertation titled **"EVALUATION OF SERUM AND SALIVARY LEPTIN CONCENTRATIONS IN PERIODONTALLY HEALTHY AND CHRONIC PERIODONTITIS INDIVIDUALS BEFORE AND AFTER NON SURGICAL PERIODONTAL THERAPY"** under our guidance and supervision in partial fulfillment of the regulations laid down by the Tamilnadu Dr.M.G.R.Medical University, Chennai-600032 for M.D.S **BRANCH-II**

Dr. S.ELANCHEZHIYAN, M.D.S.
Professor and Head
Department of Periodontics,
Vivekanandha Dental College for Women
Tiruchengode - 637205, Tamilnadu, India

Seal & Signature of H.O.D

Dr.S.ELANCHEZHIYAN M.D.S,
Professor and Head,
Department of Periodontics
Vivekanandha Dental College for Women

PRINCIPAL,
VIVEKANANDHA
DENTAL COLLEGE FOR WOMEN,
Elayampalayam - 637 205.
Tiruchengode - Tk. Namakkal Dt. Tamil Nadu

Seal & Signature of Principal

Prof.**Dr. N.BALAN, MDS.**
Principal and HOD,
Department of Oral Medicine and Radiology
Vivekanandha Dental College for Women

DECLARATION

TITLE OF DISSERTATION	EVALUATION OF SERUM AND SALIVARY LEPTIN CONCENTRATIONS IN PERIODONTALLY HEALTHY AND CHRONIC PERIODONTITIS INDIVIDUALS BEFORE AND AFTER NON SURGICAL PERIODONTAL THERAPY
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DURATION OF THE COURSE	3 Years (2015-2018)
NAME OF THE GUIDE	Dr. S.ELANCHEZHIYAN, MDS
HEAD OF THE DEPARTMENT	Dr. S.ELANCHEZHIYAN, MDS

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Head of the Department

Guide

Signature of the Candidate

Dr. S.ELANCHEZHIYAN, M.D.S.
Professor and Head
Department of Periodontics,
Vivekanandha Dental College for Women
Tiruchengode - 637205, Tamilnadu, India

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This is to certify that this dissertation work titled **"EVALUATION OF SERUM AND SALIVARY LEPTIN CONCENTRATIONS IN PERIODONTALLY HEALTHY AND CHRONIC PERIODONTITIS INDIVIDUALS BEFORE AND AFTER NON SURGICAL PERIODONTAL THERAPY"** of the candidate **Dr.P.S.VIOLA ESTHER,** with registration Number **241513503** for the award of degree **MASTER OF DENTAL SURGERY** in the branch of **PERIODONTICS**. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **3%** of plagiarism in the dissertation.

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Dr. S.ELANCHEZHIYAN, M.D.S.
Professor and Head
Department of Periodontics,
Vivekanandha Dental College for Women
Tiruchengode - 637205, Tamilnadu, India

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Introduction

Periodontitis begins as a microbial infection, followed by host-mediated destruction of soft tissue caused by hyperactivated or primed leukocytes and the generation of cytokines, eicosanoids, and matrix metalloproteinases that cause significant connective tissue and bone destruction. Cytokines, such as Interleukin- 1β, Tumor Necrosis Factor-α, prostaglandin E2, and recently leptin, have been shown to orchestrate the host response to infectious and inflammatory stimuli.[1]

In 1994, Jeffrey M. Friedman and Douglas L. Coleman discovered leptin through the study of mice at the Rockefeller University.[2]Leptin, a peptide hormone is produced predominantly by white adipose cells, although low levels have been detected in the hypothalamus, pituitary,[3] stomach,[4] skeletal muscle[5], mammary epithelia,[6] chondrocytes, placenta,[7]gastric epithelium,[8] T cells,[9] gingiva,[10] osteoblasts,[11] and in intercalated, striated, and intralobular ducts as well as in basal parts of the acini in the major salivary glands.[12]

The mature protein, encoded by the obese (ob) gene is localized into human and mouse 7 and 6 chromosomes, respectively. Zhang et al., studied the effects of leptin through mutant obese mice from the mouse colony at the Jackson Laboratory.[13]It was found that mutations in the gene encoding the protein hormone leptin "ob/ob" and receptor for leptin "db/db" were the possible cause for massively obese mice. When ob/ob mice were administered with recombinant leptin, decrease in food intake and body weight was observed.[2] Thus leptin is regarded as a ‘ Fasting signal’.

Leptin which is structurally and functionally related to the Interlukin - 6 cytokine family is a 16 kDa non-glycosylated protein, characterized by a long chain four-helical bundle structure and a pair of conserved cysteine residues to form a disulfide bridge required for full biological activity.[14]The leptin receptor Ob-R (or Lepr), is a member of the class I cytokine receptor family, which includes gp-130, the common signal transducing

receptor for the Interlukin-6 related family of cytokines. In humans, three expressions of Ob-R gene have been reported. They are Ob-Ra, Ob-Rb and Ob-Rc mRNA. Among them, Ob-Rb is abundantly expressed in the hypothalamus and controls the energy balance of the body.[15] Leptin, the 'fat sensor', not only monitors weight but also modulates glucose and lipid metabolism, thermo-genesis, neuro-endocrine function, reproduction, immunity, bone remodeling and cardiovascular function.[5]

B and T lymphocytes also express leptin receptor Ob-Rb, thereby directly regulating the B and T cell responses.[16] It activates cytokine-like signal transduction via the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway by binding to the long leptin receptor isoform (LEPRb). In addition, AMP-activated protein kinase (AMPK) is another important intracellular enzyme in leptin transduction mechanisms.[2] Modulation of the immune system by leptin is exerted at the development, proliferation, antiapoptotic, maturation, and activation levels[17] and is mediated by the regulation of hematopoiesis and lymphopoiesis.

Moreover, leptin receptors have been found in neutrophils, monocytes, and lymphocytes which suggest that leptin has a proinflammatory effect in the immune system. Leptin enhances the progression of periodontitis by mediating the promotion of T-helper 1 responses and production of other proinflammatory cytokines, such as Tumor Necrosis Factor-α, Interleukin-2, Interleukin-6.[16] Hence, it is evident that leptin plays an influential role in the mechanisms of immune response and host defense. Therefore it can be ascertained that the expression of leptin is modulated in a similar manner to the cytokine response to infectious and inflammatory stimuli in periodontitis. Despite the fact that poor periodontal health is linked to higher levels of several systemic markers, only few studies[18–20] have tried to elucidate whether successful periodontal treatment can reduce the levels of

these serological markers. In this conjecture, the present study evaluated serum and salivary leptin concentrations between healthy and patients with chronic periodontitis and to elucidate the effect of Non Surgical Periodontal Therapy (NSPT) on serum and salivary leptin concentrations in chronic periodontitis.

Aims & Objectives

AIM

i. To compare the salivary and serum leptin concentrations between healthy individuals and chronic periodontitis patients.

ii. To evaluate the effect of non surgical periodontal therapy on serum and salivary concentrations of leptin in patients with chronic periodontitis.

OBJECTIVE

- To measure the serum and salivary leptin levels in subjects with and without chronic periodontitis by ELISA.
- To assess whether NSPT can alter the salivary and serum leptin levels in chronic periodontitis.
- To correlate the salivary and serum leptin levels with clinical parameters before and after NSPT.

Review of Literature

The Biology Of Leptin

Theodore et al.,1997[21] stated that leptin is derived from the Greek word leptos, meaning "thin". It is the "hormone of energy expenditure", predominantly produced by adipose cells that helps to regulate energy balance by inhibiting hunger. Leptin is opposed by the actions of the hormone ghrelin, the "hunger hormone". Both hormones act on receptors in the arcuate nucleus of the hypothalamus to regulate appetite to achieve energy homeostasis. They pioneered the concept that adipose tissue is not an inert energy storage organ but an active endocrine organ.

Licinio et al.,2000[22] found that leptin is secreted in a pulsatile fashion and has a significant diurnal variation with higher levels in the evening and early morning hours. Circulating leptin levels reflect primarily the amount of energy stored in fat and secondarily acute changes in caloric intake. Factors promoting leptin secretion are excess energy stored as fat (obesity), Overfeeding, Glucose, Insulin, Glucocorticoids, Estrogens, Inflammatory cytokines, including Tumor Necrosis Factor-α and Interleukin-6. Factors inhibiting leptin secretion arelow energy states with decreased fat stores,fasting, catecholamines and adrenergic agonists, thyroid hormones, androgens, peroxisome, Proliferator-activated Receptor-γ (PPARγ) agonists.

Role Of Leptin In Energy Homeostasis

Elmquist JK etal.,2006[23] experimented rat brain and found thatleptin mediates its effects by binding to specific leptin receptors (ObRs) expressed in the brainas well as in peripheral tissues. Alternative splicing generates several isoforms of ObRs. TheObRa isoform (the short leptin receptor isoform) is thought to play an important role intransporting leptin across the blood–brain barrier. The ObRb isoform (the long leptinreceptor isoform)

mediates signal transduction and is strongly expressed in the hypothalamus,an important site for the regulation of energy homeostasis and neuroendocrine function.

Robertson SA et al.,2008[24] interpreted that the circulating leptin level serves as a gauge for energy reserves and directs the central nervoussystem to adjust food intake and energy expenditure accordingly. Leptin exerts immediateeffects by acting on the brain to regulate appetite via ObRb-receptor binding in thehypothalamus.Leptin activates a complex neural circuit comprising of anorexigenic (i.e.appetite-diminishing) and orexigenic (i.e. appetite-stimulating) neuropeptides to control foodintake. Outside of the hypothalamus, leptin interacts with the mesolimbic dopamine system and nucleus of the solitary tractof the brainstem to contribute to satiety.It not only signals the central nervous system to decrease food intake, but alsoincrease energy expenditure.

Roth JD et al.,2009[25] found that patients with congenital leptin deficiency due to mutations in the leptin gene orextreme leptin resistance due to mutations of the leptin receptor gene are obese due to marked hyperphagia. For patients with leptin deficiency, administering leptin in replacementdoses reduce food intake via neural circuits that diminish the perception of food reward andenhance the response to satiety signals and normalizes body weight. However, leptinadministration at pharmacologic doses to the vast majority of obese humans, who haverelatively high levels of leptin and are resistant to it, induces little if any weight loss.Thus, accumulating evidence suggests that leptin is physiologically more important as anindicator of energy deficiency and as a possible mediator of adaptation to starvation.

Leptin In Saliva

De Matteis R et al.,2010[26]carried out a detailed study of human salivary glands as potential leptin-producing organs. Biopsiesof salivary glands (submandibular and parotid) obtained from male and female patients during surgery for differentclinical indications were subjected to immunohistochemical study for the presence of leptin, its functional receptor,insulin and glucagon. Double immunohistochemical staining (silver–gold intensification and avidin–biotin–peroxidase) wasused for the visualization of glucagon and leptin labelling, respectively. The results showed that intralobularduct cells of submandibular and parotid glands are immunoreactive for leptin, leptin receptor and glucagonbut not for insulin.

Bohlender J et al.,2014[27] found leptin distributed throughout themajor salivary glands with obvious intracellular concentrationsin granula. In contrast, immunostaining for theleptin receptor was found exclusively in the membranes ofthe glandular cells. A high density of the leptin receptorwas localised in the epithelia of the duct lumen. PCRanalysis proved the autonomous expression of leptin bythe salivary glands independently from adipocytes.In the light of recent findings ofleptin influencing the growth of rodent salivary glands, thepresence and distribution of leptin and its receptor suggestsan autocrine role of salivary leptin within the glands.

Lucena S et al.,2016[28]assessed if the induction of increased levels of circulating leptin influence the immunohistochemical expression of leptin at the level of major salivary glands in Wistar rats. It was found that the expression, in qualitative terms of leptin has been positive, being more evident in submandibular and sublingual glands, either in the acini or ducts. The results suggest that circulating leptin levels may not affect the expression of this hormone in the major salivary glands.

Role Of Leptin In Immunity

G.Matarese et al.,2000[17] indicated that the primary amino acid sequence of leptin belong to the long-chain helical cytokine family, such as IL-2, IL-12, and IL-6. In fact, leptin receptor (Ob-R) shows sequence homology to members of class I cytokine receptor (gp130) superfamily that includes the receptor for IL-6, leucocyte inhibitory factor (LIF), and granulocyte colony-stimulating factor (G-CSF). Moreover, Ob-R has been shown to have the signaling capabilities of IL-6-type cytokine receptors, activating JAK-STAT, PI3K, and MAPK signaling pathways. In this context, a role for leptin in the regulation of innate immunity has been proposed. Consistent with this role of leptin in the mechanisms of immune response and host defense, circulating leptin levels are increased upon infectious and inflammatory stimuli such as LPS, turpentine, and cytokines.

C. Mart´ın-Romero et al.,2000[29] demonstrated that Human leptin alone is not able to activate human peripheral blood lymphocytes in vitro even though leptin receptor is present and activated in T lymphocytes. However, when T lymphocytes are costimulated with phytohaemagglutinin(PHA) or concanavalin A (Con A), leptin dose-dependently enhances the proliferation and activation of cultured T lymphocytes, achieving maximal effect at 10 nm concentration. However, when maximal concentrations of PHA or Con A are employed, leptin has no further effect. These effects of leptin on T lymphocytes are observed even in the absence of monocytes, suggesting a direct effect of human leptin on circulating T lymphocytes when they are costimulated.

F.Maingrette et al.,2003[30] assesed leptin regulating monocyte function in vitro experiments measuring free radical production and found leptin was shown to stimulate the oxidative burst in monocytes and binding of leptin at the macrophage cell surface increases lipoprotein lipase expression through oxidative stress and protein kinase-C (PKC) dependent

pathways. Leptin at 1 ng/ml, mediates the inflammatory infiltrate and induces tissue factor expression in human peripheral blood mononuclear cells. On the other hand, human leptin seems to downregulate oxidative burst in previously activated monocytes.

C. K. Wong et al.,2007[31] explained that leptin could upregulate cell surface expression of adhesion molecules ICAM-1 and CD18 on eosinophils but suppress ICAM-3 and L-selectin. Moreover, leptin could also stimulate the chemokinesis of eosinophils and induce the release of inflammatory cytokines IL-1β and IL-6 and chemokines IL-8, growth-related oncogene-alpha, and monocyte chemoattractant protein-1.

K. Claycombe et al.,2008[32] found that Leptin signaling deficiency impairs humoral and cellular immunity. The leptin receptor Ob-Rb is expressed by B and T lymphocytes, suggesting that leptin regulates directly the B and T cell responses. The leptin modulation of the immune system is also mediated by the regulation of hematopoiesis and lymphopoiesis. Thus, seven days of provision of recombinant leptin promoted substantial lymphopoiesis, with a twofold increase of the numbers of B cells in the marrow of obese mice while doubling and tripling, respectively, the numbers of pre-B and immature B cells. Twelve days of supplementation brought these subpopulations to near-normal proportions. Leptin treatment also facilitated myelopoiesis such that the marrow of the obese mice contained normal numbers of monocytes and granulocytes after 7 days.

N. Kiguchi et al.,2009[33] conducted an in vitro study and found that human leptin stimulating proliferation and activation of human circulating monocytes, promoting the expression of activation markers: CD69, CD25, CD38, and CD71, in addition to increasing the expression of monocytes surface markers, such as HLA-DR, CD11b andCD11c. Besides, leptin potentiates the stimulatory effect of LPS on the proliferation and activation of human monocytes. Moreover, leptin dose-dependently stimulates the production of TNF-

α and IL-6 and enhances chemokine ligand expression in cultured murine macrophage, through activation of a JAK2-STAT3 pathway.

B. Mattioli et al.,2009[34] stated that Leptin induces functional and morphological changes in human dendritic cells, directing them towards Th1 priming and promoting DC survival via the phosphoinositide 3 kinase (PI3K) signaling pathway. The involvement of leptin signaling in dendritic cell survival and maturation has been observed in leptin receptor (Ob-R) deficient db/db mice. db/db mice displayed markedly reduced expression of costimulatory molecules and a Th2-type cytokine profile, with poor capacity to stimulate allogenic T cell proliferation. Thus, leptin has also been found to decreases MIP-1-α production by dendritic cells. Similar to leptin effect on monocytes, it may increase the survival of dendritic cells, and it may also increase the expression of surface molecules, such as CD1a, CD80, CD83, or CD86.

The Role Of Leptin In Systemic Diseases

Karmazyn et al.,2008[35] described some of the cardioprotectiveeffects of leptin which included reduced extent of myocardialinfarction (MI) and protection against reperfusiondamage by local autocrine effects (the heart itself alsoproduces leptin) probably mediated through nitric oxide and alsoantilipotoxic effects.

Seufert J et al.,2010[36] examined pancreatic islets isolated from pancreas of human donors for their responses to leptin. The presence of leptin receptors on islet β-cells was demonstrated by double fluorescence confocal microscopy. They suggested that desensitization of leptin reception at the levels of the hypothalamus and the pancreatic β-cells resulted in hyperphagia and hyperinsulinemia, respectively which may be important factors in the pathogenesis of adipogenic diabetes mellitus.

Menha Swellam et al.,2012[37] assessed the role of soluble leptinand LepRb in non alcoholic fatty liver disease (NAFLD) and investigated whether leptin receptorgene (LepR) single nucleotide polymorphism influences NAFLD complicated with or without type 2 diabetes mellitus(T2DM). Blood samples from 90 obese NAFLD cases and 30lean controls of matched age and sex were recruited in the study.Among the NAFLD patients, 43 were T2DM. Mutant LepRgenotype were significantly higher in mild–severesteatosis and in NAFLD with type 2 diabetes mellitus when compared with mild steatosisand those without T2DM. Elevatedleptin level seems to be a feature of steatosis, and it appears toincrease as hepatocyte steatosis develops. Moreover, polymorphismof LepR gene contributes to the onset of NAFLD by regulating lipidmetabolism and affecting insulin sensitivity.

Manole et al.,2013[38] leptin stated that leptin is involved in the inductionand progression of experimental autoimmune encephalomyelitis (EAE). The immunomodulatoryeffects of leptin have also been linked to enhanced susceptibility to other autoimmunedisease such as multiple sclerosis.They suggested that increased leptin secretion occurs in acute phases of multiple sclerosis and correlates with CSF production of IFN.

VanSaun et al.,2015[39] found that tumor-associated leptin receptor levels contribute to tumor growth and progression. Increaseddetection of ObR in ovarian cancers was correlated withdecreased survival. Three single-nucleotide polymorphisms in the leptin receptorgene (K109R, K656N, and Q223R) showed an associationwith increased basal-like breast cancer risk. Theseresults suggest that tumor leptin receptor levels directlyinfluence growth and progression.

Smekal et al.,2017[40] found that chronic intravenous administration of leptin increasesheart rate and mean arterial blood pressure by activationof the sympathetic nervous system and increased releaseof catecholamines. Another mechanism described in the

development of hypertension in which leptinhas been found to decrease diuresis and increased sodiumreuptake in the kidneys. Elevated leptin levels are associatedwith myocardial infarction and stroke, independentlyof traditional risk factors or obesity and are associatedwith calcification of the coronary arteries.

Materials & Methods

A case control and quasi experimental study which involved patients visiting the Department of Periodontics in Vivekanandha Dental College for women was conducted. The study was approved by the Institutional ethics committee. Before the commencement of the study, it was clearly explained and informed consent was obtained from all patients.

A total of 60 participants with normal Body Mass Index (18.5-24.9 kg/m^2)[41]were enrolled in the study.Patients with chronic periodontitis were diagnosed based on the American Academy of Periodontology Task Force Report on the Update to the 1999 Classification of Periodontal Diseases and Conditions.[42] They were divided into three groups based on the following criteria.

INCLUSION CRITERIA

GROUP A: Healthy Volunteers (n=26)

- Systemically and periodontally healthy individuals
- Good oral hygiene
- Probing depth (PD) <3mm
- No clinical attachment loss (CAL)
- Plaque index[44] [PI] <1
- Gingival index[45] [GI] <1

Group B: Chronic Periodontitis before treatment (n=30)

- Patients with signs of gingival inflammation
- Presence of bleeding on probing
- Clinical Attachment Loss > 4mm
- PD>4mm
- Radiographic evidence of bone loss

Group B1: Chronic Periodontitis after treatment (n=30)

Group B patients underwent non surgical periodontal therapy and revaluated after 3 months

EXCLUSION CRITERIA:

- Use of tobacco in any form
- Alcoholism
- Pregnancy
- Presence of any gross pathology and systemic disease.
- Any periodontal therapy in the past 12 months
- Aggressive Periodontitis
- Any antimicrobial, anti inflammatory therapy
- Immunosuppressive therapy for the past 6 months

Sociodemographic characteristics of the study population:

This included bio social and metabolic variables, frequency of tooth brushing and number of teeth present in the oral cavity. Biosocial variables comprise of age, gender and socio-economic status (SES). Metabolic variable included measurement of BMI. All the informations were obtained by questioning the participants verbally. All participants were in the age group of 31 to 65 years. There was almost equal distribution of males and females. Group A (healthy) comprised of 13 males and 13 females. Group B (Chronic Periodontitis) comprised of 16 males and 14 females. Socioeconomic status was assessed by Modified Prasad classification[43] as follows:

Class 1 - ≥ Rs 5156

Class 2 - Rs 2578 –5155

Class 3 - Rs 1547 –2577

Class 4- Rs 773–1546

Class 5- ≤ Rs773

BMI was calculated based on each subject's weight in kilograms divided by the square of his height in meters (kg/m^2). Participants with normal weight classified based on WHO recommendations[41] were included in the study.

<16 kg/m^2 -severe underweight

16·0–16·9 kg/m^2 -moderate underweight

17·0–18·49 kg/m^2 -mild underweight

18·5–24·9 kg/m^2 -normal range

≥25 kg/m^2 -overweight

25–29·9 kg/m^2 -preoboese

≥30 kg/m^2 -obesity

30–39·9 kg/m^2 - obese class I

35–39·9 kg/m^2 -obese class II

≥40 kg/m^2 - obese class III

Clinical examination:

A thorough medical, dental, personal history was obtained from each patient. All the clinical parameters were examined after collection of clinical samples in order to avoid contamination of the samples at baseline and at recall visits. For each patient, Turesky et al 1970 plaque index (PI),[44] Loe and Silness 1975 gingival index (GI),[45] Ainamo and Bay 1975 Gingival Bleeding Index (GBI),[46] probing depth (PD) and clinical attachment loss (CA loss) were recorded at baseline and after 3 months.

Turesky et al Plaque Index, 1970:

Disclosing agent was applied on both buccal and lingual surfaces and patient was advised to rinse with water. Scores were recorded based on the following criteria.

Scores	Criteria
0	No plaque
1	Separate flecks of plaque at the cervical margin of the tooth
2	A thin continuos band of plaque (up to one mm) at the cervical margin of the tooth
3	A band of plaque wider than one mm but covering less than one-third of the crown of the tooth
4	Plaque covering at least one-third but less than two-thirds of the crown of the tooth
5	Plaque covering two-thirds or more of the crown of the tooth

Loe and Silness Gingival Index, 1963

The severity of gingivitis is scored on distal facial papilla, facial margin, mesial facial papilla, entire lingual margin of selected teeth.

Score	Criteria
0	Normal gingiva.
1	Mild inflammation- - slight change in color, slight edema. No bleeding on probing.
2	Moderate inflammation -- redness, edema and glazing. Bleeding on probing.
3	Severe inflammation -- marked redness and edema. Tendency to spontaneous bleeding. Ulceration.

Ainamo and Bay Gingival Bleeding Index,1975:

It is performed through gentle probing of the orifice of the gingival crevice by No 23 explorer. If bleeding occurs within 10seconds, positive finding is recorded and then expressed as a percentage of the number of sites examined.

Probing depth and Clinical Attachment Level:

Pressure sensitive probe calibrated in millimeters was used to examine six sites (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual) on each tooth excluding the third molars. Probing depth is measured from gingival margin to base of the pocket and Clinical attachment level is measured from cementoenamel junction to base of the pocket.

Collection of clinical samples

Clinical samples (saliva and blood) were collected at baseline and after 3 months.The patients were asked to abstain from eating and drinking (except for water) for two hours before collecting samples. They were made to sit comfortably in an upright position on the dental chair

Collection of saliva

The subjects were advised to rinse his or her mouth with distilled water and then to relax for five minutes. Whole unstimulated saliva (4 ml) was collected by modified draining method as depicted in figure-1. They were asked to expectorate into disposable polypropylene tubes fitted with a funnel for ease of collection every 30 s over a period of 5 min. Saliva samples were immediately centrifuged at 3000 rpm for 5 min to remove the cell debris and 2ml of the supernatant was pippetted out in 5 ml aliquots and stored at -80^0C until analysis.

Collection of serum

Four milliliters of blood was collected from the ante cubital fossa by venipuncture using 20 gauge needles with a 5 ml syringe as depicted in figure-2. Blood sample was allowed to clot at room temperature and after 1 hour, serum was extracted from blood by centrifuging at 3000rpm for 5 min and 2 ml of the extracted serum was transferred to 5ml aliquots and stored at -80^0C (as shown in figure-4) till the time of assay.

Non Surgical Periodontal Therapy

Non-surgical periodontal treatment (NSPT) included scaling and root planing (SRP) and institution of oral hygiene instructions which was performed with the help of ultrasonic scalers(Woodpecker) and hand curettes (Hu-Friedy curretes, Chicago, IL, U.S.A) for patients with chronic periodontitis . Scaling was done on the day of initial collection of samples and root planning was completed within 2 weeks after the enrollment of the participants in the study. All patients were instructed to perform modified Bass method of brushing twice in a day and dental flossing.They were refrained from mouthwashes, antimicrobials during the course of study. They were contacted via telephone once in 15 days to ensure whether they follow oral hygiene instructions properly

Analysis of leptin by ELISA:

The RayBio® Human Leptin ELISA kit, GA, USA was used to quantify the serum and salivary leptin concentrations. The following reagents and materials are used.

Reagents used:

Component	Size / Description
Leptin Microplate (Item A)	96 wells (12 strips x 8 wells) coated with anti-Human Leptin.
Wash Buffer Concentrate (20X) (Item B)	25 ml of 20X concentrated solution.

Standard Protein (Item C)	2 vials of Human Leptin. 1 vial is enough to run each standard in duplicate.
Detection Antibody Leptin (Item F)	2 vials of biotinylated anti-Human Leptin. Each vial is enough to assay half the microplate.
HRP-Streptavidin Concentrate (Item G)	200 µl 160X concentrated HRP-conjugated streptavidin.
TMB One-Step Substrate Reagent (Item H)	12 ml of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution.
Stop Solution (Item I)	8 ml of 0.2 M sulfuric acid.
Assay Diluent A (Item D)	30 ml of diluent buffer, 0.09% sodium azide as preservative.
Assay Diluent B (Item E)	15 ml of 5X concentrated buffer.

Additional materials

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

2. Precision pipettes to deliver 2 µl to 1 ml volumes.

3. Adjustable 1-25 ml pipettes for reagent preparation.

4. 100 ml and 1 liter graduated cylinders.

5. Absorbent paper.

6. Distilled or deionized water.

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

8. Tubes to prepare standard or sample dilutions.

Reagent preparation

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

2. Assay Diluent B (Item E) was diluted 5-fold with deionized or distilled water before use.

3. Sample dilution: Assay Diluent A (Item D) was used for dilution of serum samples. 1X Assay Diluent B (Item E) was used for dilution of cell culture supernatant samples.

4. Preparation of standard: 440 µl Assay Diluent A (for serum/ samples) or 1X Assay Diluent B (for salivary samples) was added into Item C vial to prepare a 220 ng/ml standard. The powder was dissolved thoroughly by a gentle mix. 2µl Leptin standard was added from the vial of Item C, into a tube with 1098 µl Assay Diluent A or 1XAssay Diluent B to prepare a 400 pg/ml stock standard solution. 300 µl Assay Diluent A or 1X Assay Diluent B was pipetted into each tube. Stock standard solution was used to produce a dilution series as shown below. Each tube was mixed thoroughly before the next transfer. Assay Diluent A or 1X Assay Diluent B served as the zero standard (0 pg/ml).

		Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Zero standard
Diluent Volume	Item C+ 440 µl	1098 µl	300µl	300µl	300µl	300µl	300µl	300µl	300µl
Conc.	220 ng/ml	400 pg/ml	160 pg/ml	64 pg/ml	25.6 pg/ml	10.24 pg/ml	4.1 pg/ml	1.64 pg/ml	0 pg/ml

5. 20 ml of Wash Buffer Concentrate (20X) (Item B) was diluted into distilled water to yield 400 ml of 1X Wash Buffer.

6. Detection Antibody vial (Item F) was spinned before use. 100 µl of 1X Assay was added to Diluent B (Item E) into the vial to prepare a detection antibody concentrate and pipetted up and down to mix gently. The detection antibody concentrate was diluted 80-fold with 1X Assay Diluent B (Item E).

7. The HRP-Streptavidin concentrate vial (Item G) was spinned and pipetted up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate was diluted 160-fold with 1X Assay Diluent B (Item E).

Assay procedure

1. 100 µl of each standard and sample was added into appropriate wells. Wells were covered and incubated for 2.5 hours at 36^0C with gentle shaking.

2. The solution was discarded and washed 4 times with 1X Wash Solution. Each well was filled with wash Buffer (300 µl) using a Pipette. After the last wash, remaining wash Buffer was aspirated. The plate was inverted and blotted against clean paper towels.

3. 100 µl of 1X prepared biotinylated antibody was added to each well and incubated for 1 hour.

4. The solution was discarded and washed as decribed above.

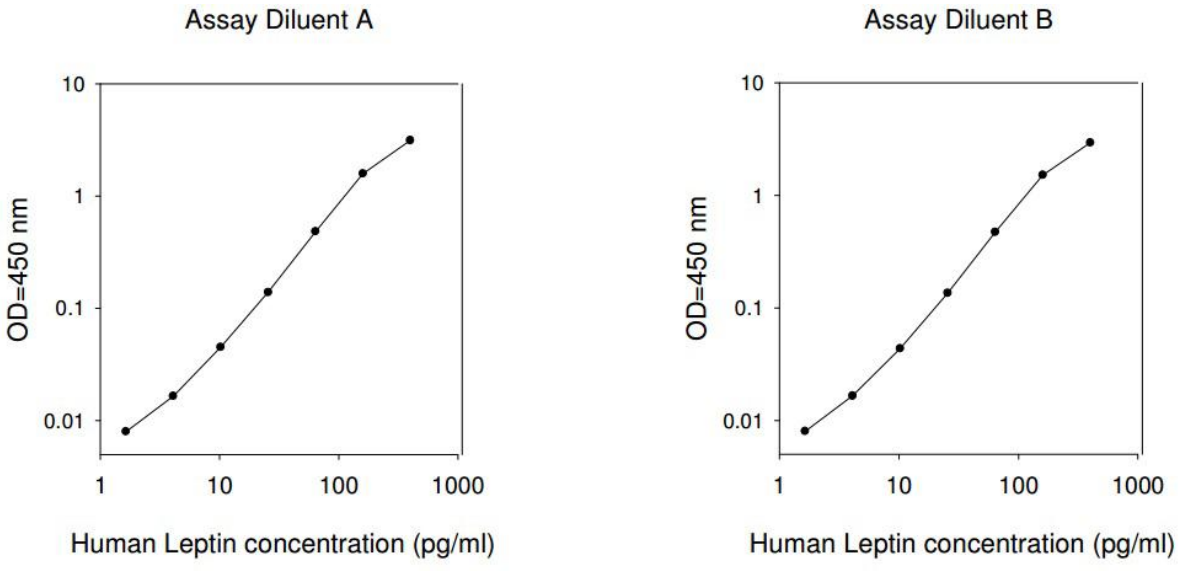
5. 100 µl of prepared Streptavidin solution was added to each well and incubated for 45 minutes

6. The solution was discarded and washed again

7. 100 µl of TMB was added to One-Step Substrate Reagent (Item H) to each well and incubated for 30 minutes.

8. 50 µl of Stop Solution (Item I) was added to each well and read at 450 nm immediately.

Standard curve:



Sensitivity

The minimum detectable dose of Human Leptin was determined to be 1 pg/ml.

Specificity

The Leptin ELISA kit shows no cross-reactivity with any of the cytokines tested: Human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-7, IL- 8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, I-309, IP-10, G-CSF, GM-CSF, IFN gamma, MCP- 1, MCP-2, MCP-3, MDC, MIP-1 alpha, MIP-1 beta, MIP-1 delta, PARC, PDGF, RANTES, SCF, TARC, TGF-beta, TIMP-1, TIMP-2, TNF-alpha, TNF-beta, TPO, VEGF.

APPENDIX - 1

PROFORMA

Dissertation title: Evaluation of salivary and serum leptin concentrations in healthy and chronic periodontitisindividuals before and after non surgical periodontal therapy.

Name : Date:

Age/Sex: O.P.No :

Address: Income :

HISTORY

Chief Complaint:

Past Medical History:

Past Dental History:

Personal History:

General Examination:

Height :

Weight:

BMI :

Intra Oral Examination:

No of teeth

INDICES

BASELINE

PLAQUE INDEX (Turesky et al 1970) :

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

Score:

Interpretation :

GINGIVAL INDEX (Loe&Silness 1963)**:**

16	12	24	36	32	44

Score:

Interpretation:

GINGIVAL BLEEDING INDEX (Ainamo &Bay 1975)

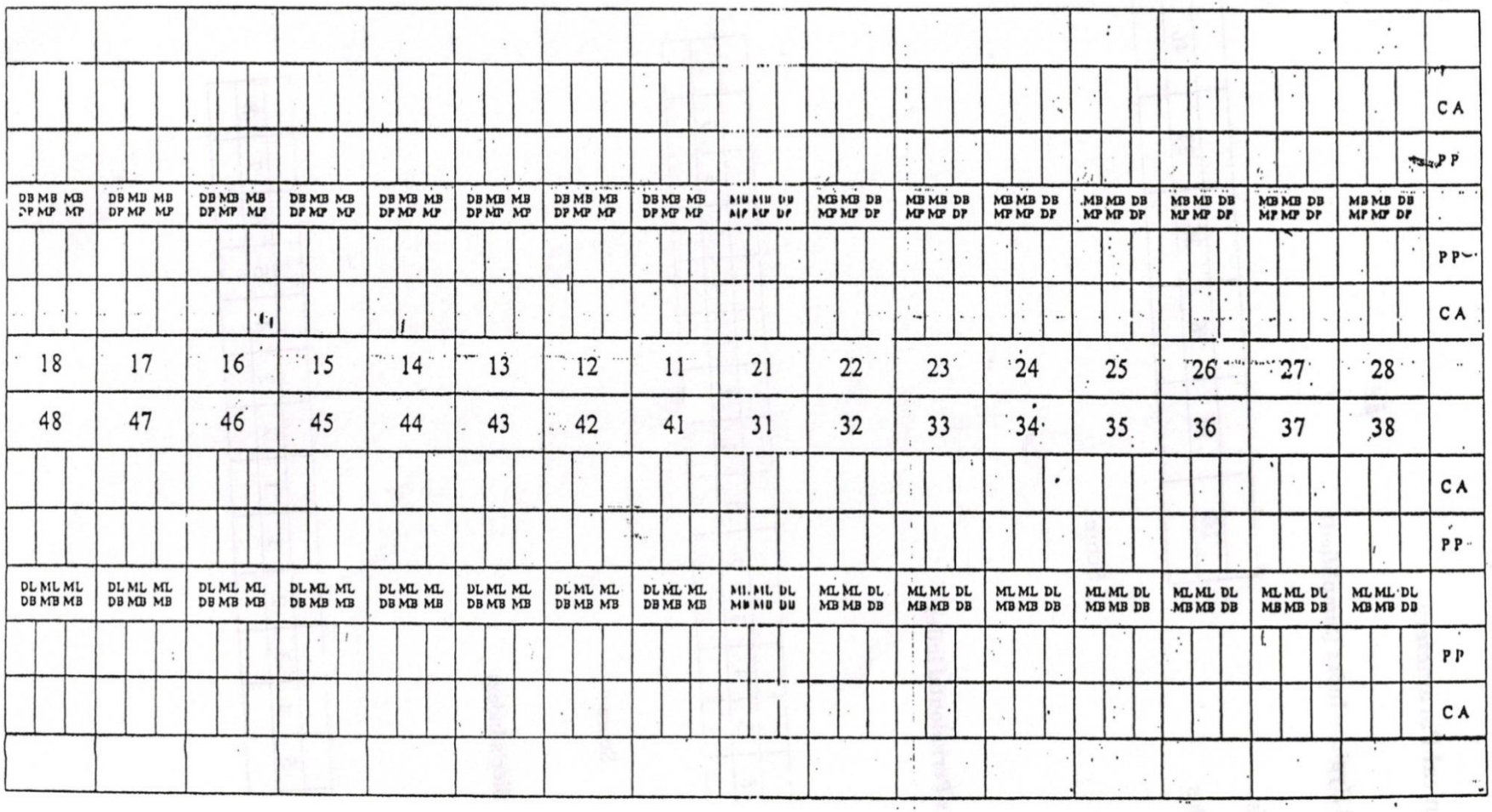
17	16	15	14	13	12	11	21	22	23	24	25	26	27

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

No of sites:

Percentage of sites:

Measurement of the Periodontal Pocket and Clinical Attachment



RADIOGRAPHIC FINDINGS:

DIAGNOSIS:

INDICES

3 MONTHS

PLAQUE INDEX (Turesky et al 1970) :

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

Score:

Interpretation :

GINGIVAL INDEX (Loe&Silness 1963)**:**

16	12	24	36	32	44

Score:

Interpretation:

GINGIVAL BLEEDING INDEX (Ainamo &Bay 1975):

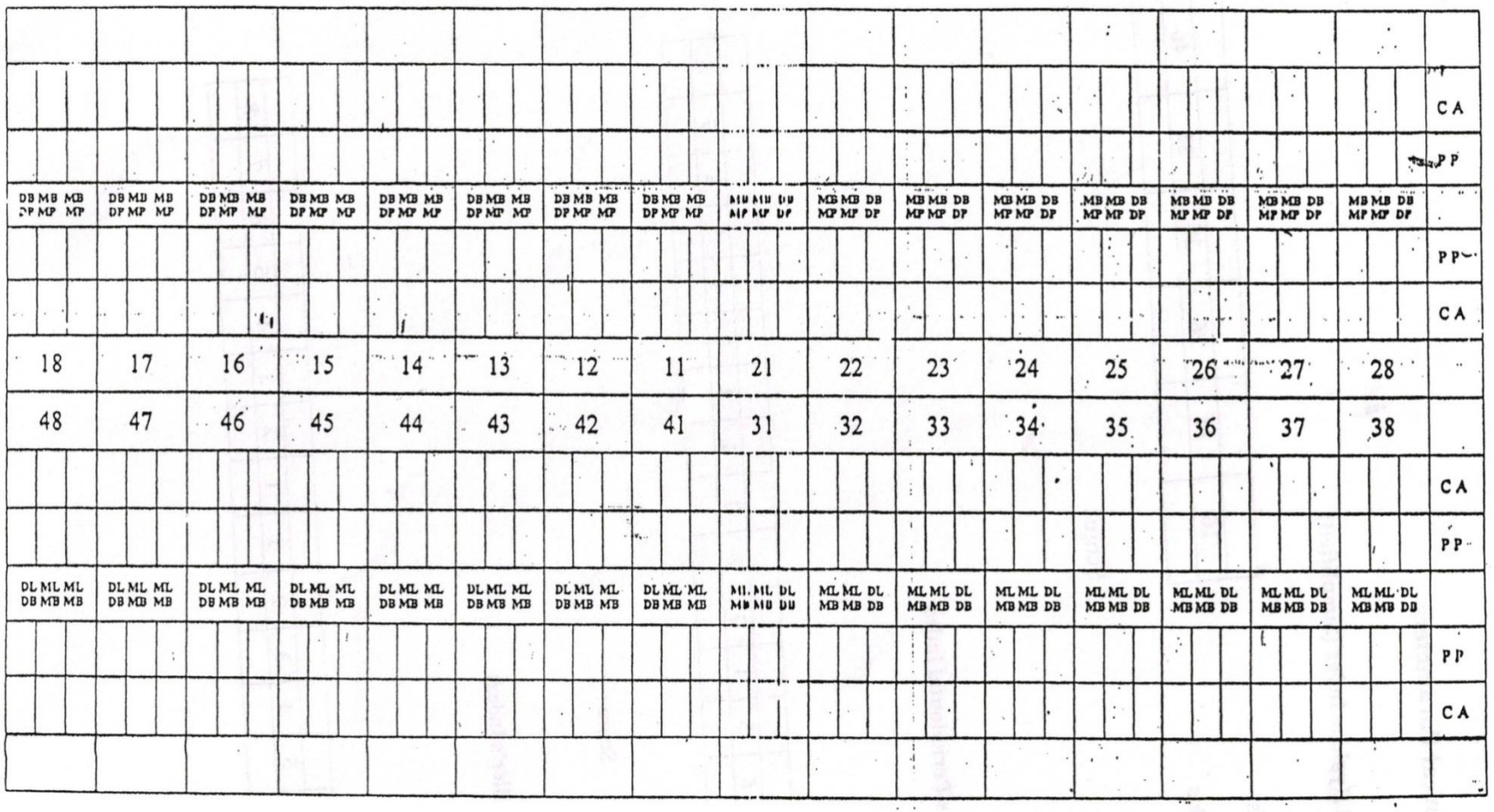
17	16	15	14	13	12	11	21	22	23	24	25	26	27

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

No of sites:

Percentage of sites:

Measurement of the Periodontal Pocket and Clinical Attachment



LEPTIN ANALYSES

	BASELINE	AFTER 3 MONTHS
Serum leptin level		
Salivary leptin level		

INFORMED CONSENT FORM

விவேகானந்தா பல் மருத்துவகல்லூரி
திருச்செங்கோடு

ஈறுநோய் சிகிச்சை பிரிவு
ஒப்புதல் படிவம்

பெயர் - ஆண் / பெண் -
முகவரி - வயது-

.....ஆகிய எனக்கு பரிசோதனை பற்றிய அனைத்து விவரங்களும் தெளிவாக எடுத்துரைக்கப்பட்டது. என் எல்லா சந்தேகங்களுக்கும் பதில் அளிக்கப்பட்டன. என் எச்சில் மாதிரி மற்றும் இரத்தமாதிரி எடுப்பதற்கும் பரிசோதனைகளுக்கும் முழுமனதோடு ஒப்புக்கொள்கிறேன்.

கையொப்பம்
தேதி

APPENDIX – 2

ARMAMENTARIUM

MATERIALS AND INSTRUMENTS USED FOR CLINICAL STUDY

- Gloves
- Mouth mask
- Patient apron
- Chair apron
- Head cap
- Sterile cotton rolls
- Gauze
- Saline
- Kidney tray
- Betadine
- Lignocaine
- Syringe
- Mouth mirror
- Explorer
- Tweezer
- Pressure sensitive probe
- Ultrasonic Scalers
- Hu – Friedy Gracey Curettes

MATERIALS AND INSTRUMENTS USED FOR IMMUNOCHEMICAL ANALYSIS

- Polypropylene tube
- Borosil funnel
- Tourniquet
- 5 ml syringe
- Centrifuge
- micropipette
- 5 ml aliquots
- Deep freezer

Photographs

ARMAMENTARIUM

Instruments used for NSPT

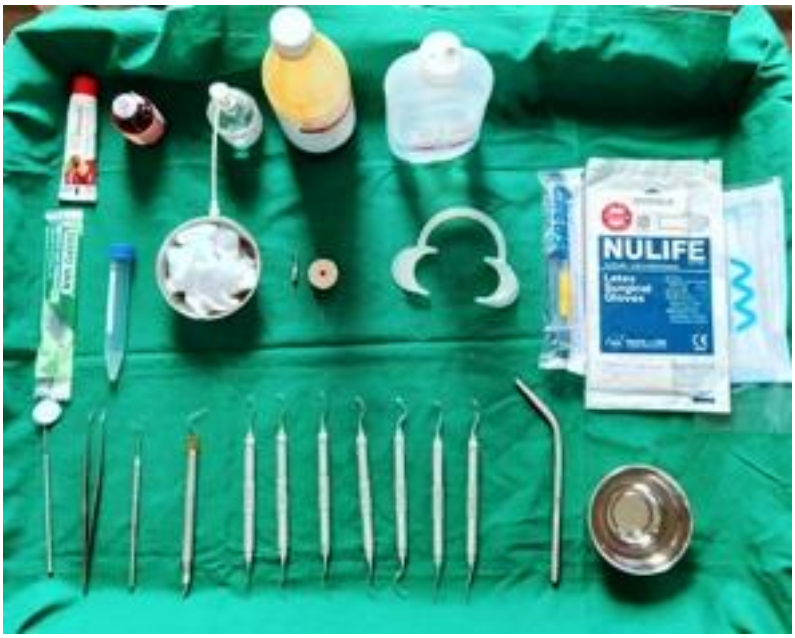


Figure -1

Contents of human leptin ELISA kit

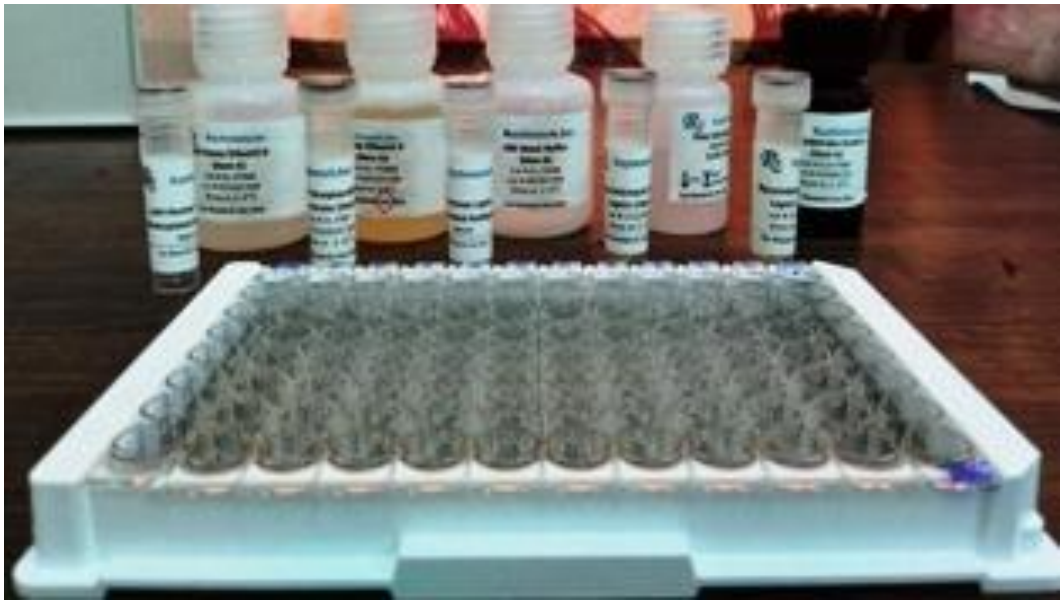


Figure-2

Measurement of PD and CAL using Pressure Sensitive Probe

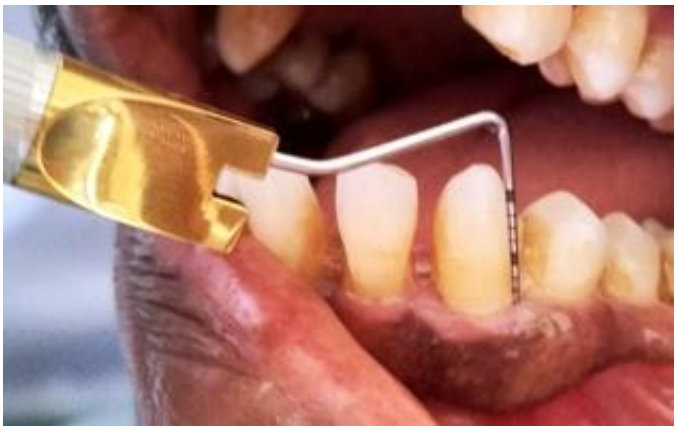


Figure -3

Preoperative View

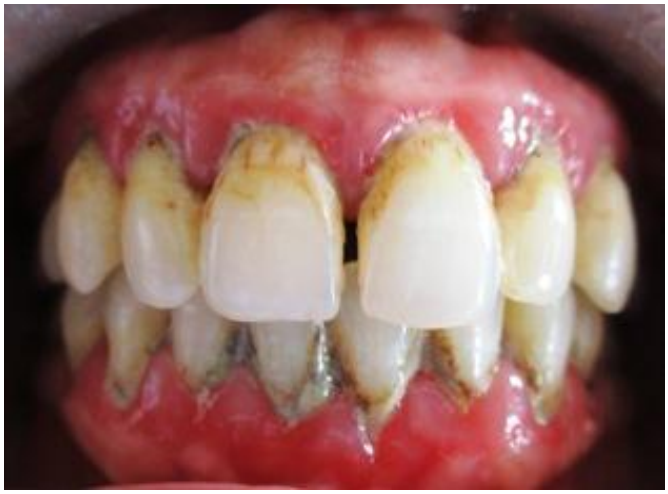


Figure - 4

Postoperative View

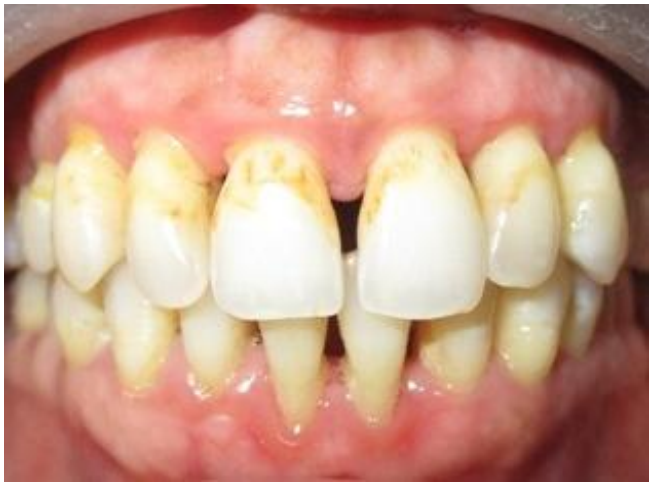


Figure – 5

COLLECTION AND STORAGE OF SAMPLES

Collection of saliva

Figure – 6

Collection of blood

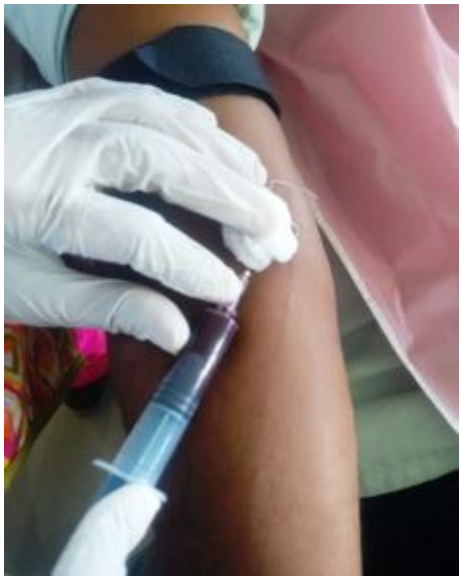


Figure - 7

Centrifugation of saliva and serum

Figure – 8

Storage of samplesat -80^0 C deep freezer

Figure - 9

ANALYSIS OF LEPTIN BY ELISA

Stored saliva and serum samples

Figure – 10

Samples showing positive for leptin

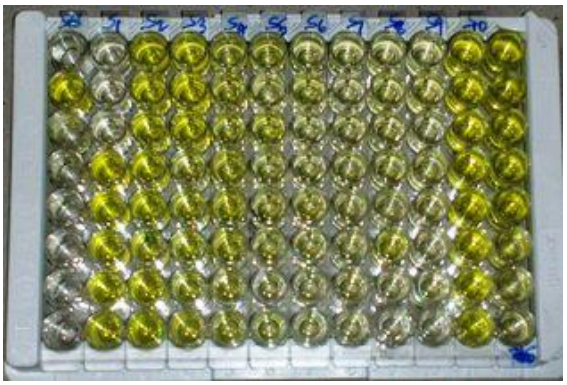


Figure – 11

Incubator used for leptin analysis

Figure – 12

ELISA reader used to assess the leptin levels

Figure - 13

Results

Statistical analysis:

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 20.0. Continuous variables were represented as mean and standard deviation (SD) and categorical variables were represented as frequency and percentage.The data was found to benormally distributed. Chi square test was used to assess sociodemographic characteristics of the study population. Intergroup comparision (between healthy and chronic periodontitis) was carried out through independent t test. Intragroup comparision (before and after NSPT) was analysed through paired t test.

Sample size calculation

The software used to calculate the sample size is GPower version 3.1.9.2. It was determined that 13 patients per group would be necessary to provide a 95% power with an alpha error of 0.05.However, based on an anticipated attrition rate of 25%, 30 patients per group were included in this study.

Sociodemographic characteristics:

In terms of age, gender, socioeconomic status, number of teeth, frequency of brushing and BMI, there was no statistically significant difference (P>0.05) determined through chi square test between group A (healthy individuals) and group B (chronic periodontitis) as depicted in table 1.

Clinical parameters:

Independent t test was used to compare the clinical parameters between healthy and chronic periodontitis. Table 2 shows patients with chronic periodontitis (group B) exhibited significantly higher scores of PI, GI, GBI, PD, CAL, % of sites with BOP, PD>4mm, CAL>5mm than healthy individuals (group A).

Comparision of Salivary and serum leptin levels between healthy and Chronic Periodontitis :

All salivary and serum samples tested positive for the leptin assay except for two salivary samples in group B. Independent t test was used for comparison of clinical samples. The mean serum leptin levels were significantly higher in patients with chronic periodontitis (288.263±64.7439 pg/ml) than healthy individuals (26.412±8.6102 pg/ml). On the contrary, mean salivary leptin levels were significantly higher in healthy individuals (10.450± 3.9180 pg/ml) than patients with chronic periodontitis (3.153± .2058 pg/ml) as depicted in table 3.

Comparision of periodontal parameters in patients with chronic periodontitis before and after NSPT by paired t test:

All the clinical parameters, reevaluated after 3 months of NSPT showed reduction in periodontitis patients. The analysis of variance showed that the differences in PI,GI, GBI, PD, CAL, % of sites with BOP, PD>4mm, CAL>4mm were found to be statistically significant ($p < 0.05$) , as depicted in table 4.

Comparison of the biochemical parameters in patients with chronic periodontitis before and after NSPT by paired t test:

Patients with chronic periodontitis underwent NSPT and salivary, serum leptin levels were revaluated after 3 months. Salivary leptin levels were significantly elevated from baseline (3.153± .2058 pg/ml) to (10.897± 3.6653 pg/ml)whereas serum leptin levels were significantly decreased from baseline (288.263±64.7439 pg/ml) to (77.330± 7.1509 pg/ml) as depicted in table 5.

Pearson's correlation among serum, salivary leptin levels and PD, CAL:

There was a significant positive correlation ($p<0.001$) among serum leptin concentrations, PD (+0.956, +0.933, +0.874) and CAL (+0.989, +0.958, +0.976) in group A, group B and group B1 but a negative significant correlation was found among salivary leptin concentration, PD (-0.960, -0.942, -0.782) and CAL (-0.846, -0.965, -0.978) in group A, group B and group C, which is shown in table 6,7,8.

Tables

TABLE 1: SOCIODEMOGRAPHIC CHARACTERISTICS OF THE STUDY POPULATION

CHARACTERISTIC	GROUP A N=26	GROUP B N=30	P VALUE
AGE	46.77±9.061	44.87±8.705	>0.05
MALES	13 (50%)	16 (53.3%)	>0.05
FEMALES	13(50%)	14(46.7%)	>0.05
SOCIO ECONOMIC STATUS CLASS I CLASS II CLASS III CLASS IV CLASS V	 13 (50.0%) 7 (42.3%) 4 (15.3%) 1 (3.8%) 1 (3.8%)	 10 (33.3%) 8 (50.0%) 7 (23.3%) 2 (6.7%) 3 (10.0%)	>0.05
NUMBER OF TEETH	29.08±2.667	29.13±2.515	>0.05
FREQUENCY OF TOOTH BRUSHING ONCE DAILY TWICE DAILY	 21 (80.8%) 5 (19.2%)	 27 (90.0%) 3 (10%)	>0.05
BMI	22.750±1.8435	22.657±1.8658	>0.05

TABLE 2:COMPARISION OF CLINICAL PARAMETERS BETWEEN HEALTHY AND CHRONIC PERIODONTITIS

CLINICAL PARAMETERS	GROUP A N=26	GROUP B N=30	MEAN DIFFERENCE	P VALUE
PLAQUE INDEX	0.388 ±.2227	2.993±.5938	-2.605	< 0.05
GINGIVAL INDEX	0.00	2.06±1.79	-2.06	< 0.05
BOP (%)	0.00	99.8	-99.80	< 0.05
PD (mm)	2.273±0.5127	5.270±0.8703	-2.997	< 0.05
CAL (mm)	1.0388±0.4879	6.1000±0.8870	-5.061	< 0.05
PD>4mm(%)	0.00	49.43±10.052	-49.43	< 0.05
CAL>5mm(%)	0.00	48.01±13.457	-48.01	< 0.05

TABLE 3: COMPARISION OF BIOCHEMICAL PARAMETERS BETWEEN HEALTHY AND CHRONIC PERIODONTITIS

BIOCHEMICAL PARAMETERS	GROUP A	GROUP B	MEAN DIFFERENCE	P VALUE
SERUM LEPTIN	26.412±8.6102	288.263±64.7439	-261.852	< 0.05
SALIVARY LEPTIN	10.450± 3.9180	3.153± .2058	7.297	< 0.05

TABLE 4: COMPARISION OF CLINICAL PARAMETERS BEFORE AND AFTER NSPT

CLINICAL PARAMETERS	GROUP B N=30	GROUP B1 N=30	T	P VALUE
PLAQUE INDEX	2.993±.5938	1.63± .396	10.476	< 0.05
GINGIVAL INDEX	2.06±1.79	1.111± .2030	23.164	< 0.05
BOP (%)	99.8	19.80± 13.428	32.554	< 0.05
PD (mm)	5.270±0.8703	2.393± .6736	27.480	< 0.05
CAL (mm)	6.1000±0.8870	3.093± .6938	38.600	< 0.05
PD>4mm(%)	49.43±10.052	29.93± 10.359	31.869	< 0.05
CAL>5mm(%)	48.01±13.457	29.63± 7.449	13.809	< 0.05

TABLE 5: COMPARISION OF BIOCHEMICAL PARAMETERS BEFORE AND AFTER NSPT

BIOCHEMICAL PARAMETERS	GROUP B	GROUP B1	T	P VALUE
SERUM LEPTIN	288.263±64.7439	77.330± 7.1509	17.747	< 0.05
SALIVARY LEPTIN	3.153± .2058	10.897± 3.6653	-10.959	< 0.05

TABLE 6: PEARSON'S CORRELATION FOR GROUP A

		PD (mm)	CAL (mm)
Serum leptin (pg/ml)	r	0.956(**)	0.989(**)
	P	<0.001	<0.001
Salivary leptin (pg/ml)	r	-0.960(**)	-0.846(**)
	P	<0.001	<0.001

TABLE 7: PEARSON'S CORRELATION FOR GROUP B

		PD (mm)	CAL (mm)
Serum leptin (pg/ml)	r	0.933(**)	0.958(**)
	P	<0.001	<0.001
Salivary leptin (pg/ml)	r	-0.942(**)	-0.965(**)
	P	<0.001	<0.001

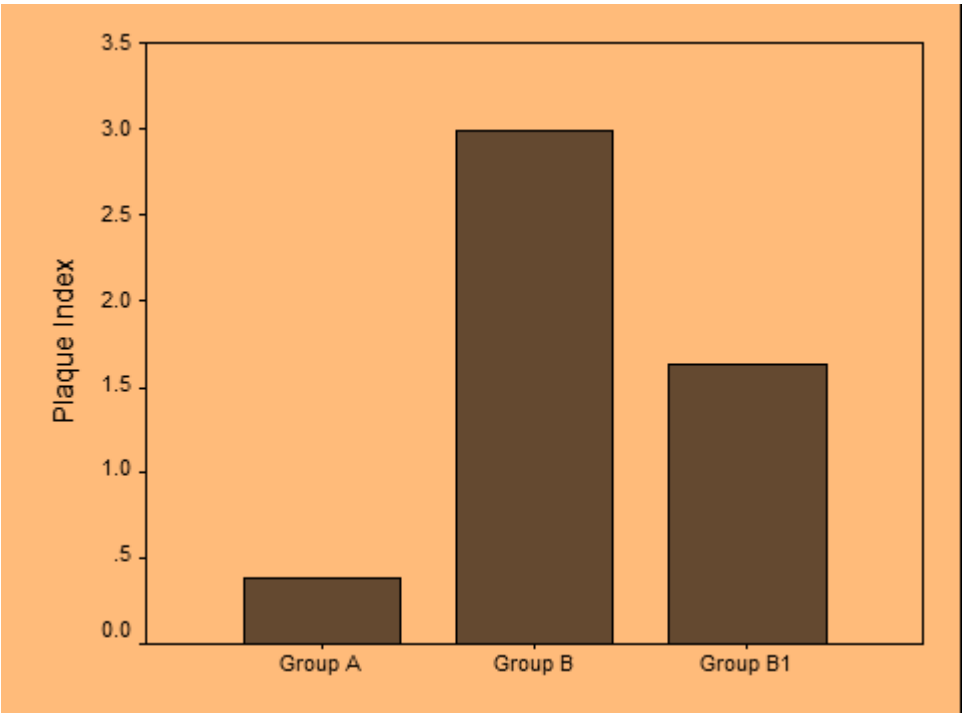
TABLE 8: PEARSON'S CORRELATION FOR GROUP B1

		PD (mm)	CAL (mm)
Serum leptin (pg/ml)	r	0.874(**)	0.976(**)
	P	<0.001	<0.001
Salivary leptin (pg/ml)	r	-0.782(**)	-0.978(**)
	P	<0.001	<0.001

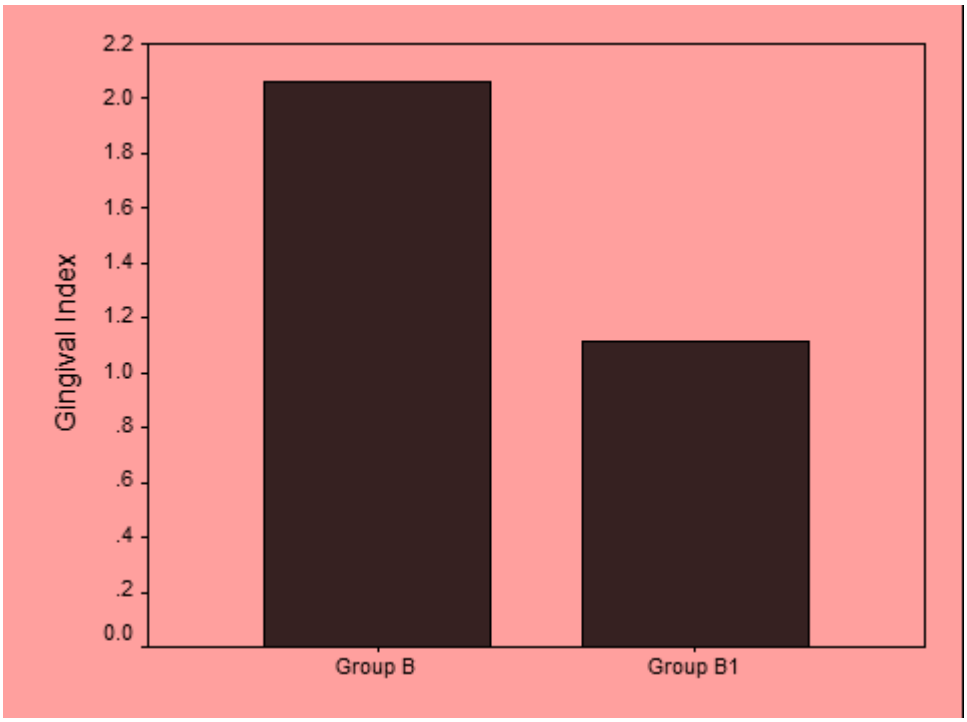
** - highly significant

Graphs

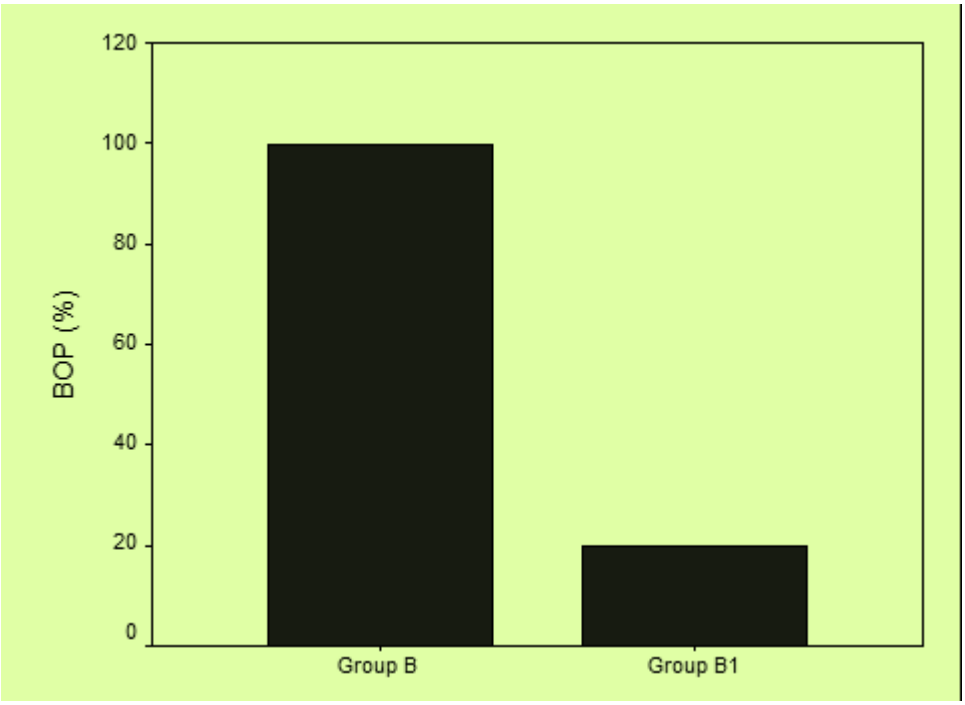
Graph – 1: Comparision of Plaque Index among healthy,CP before & after NSPT



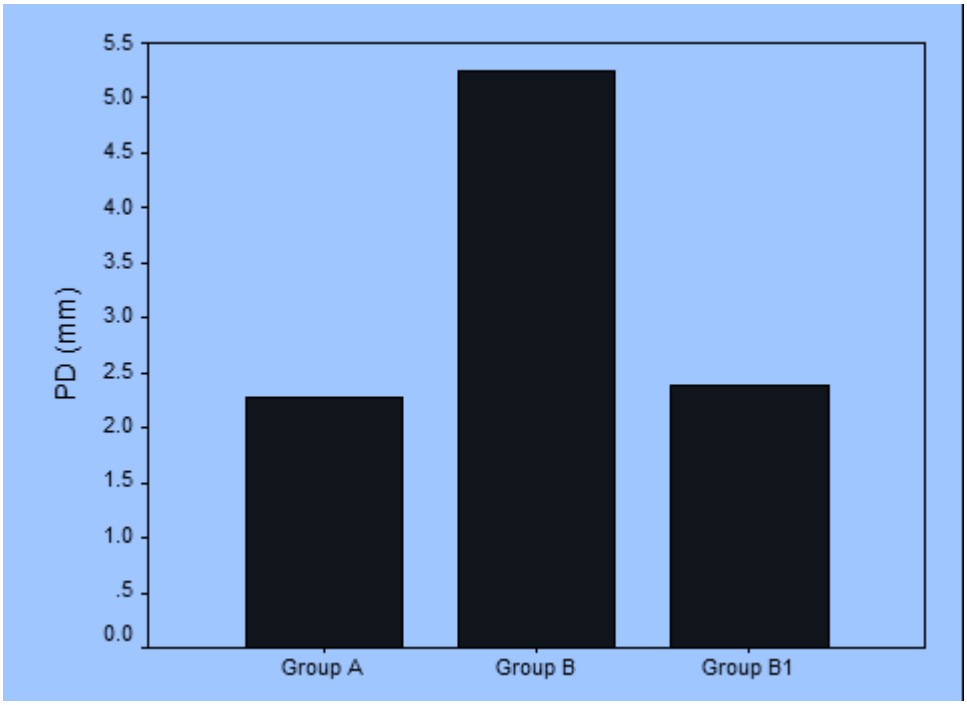
Graph - 2: Comparision Of Gingival Index In CP Patients before and after NSPT



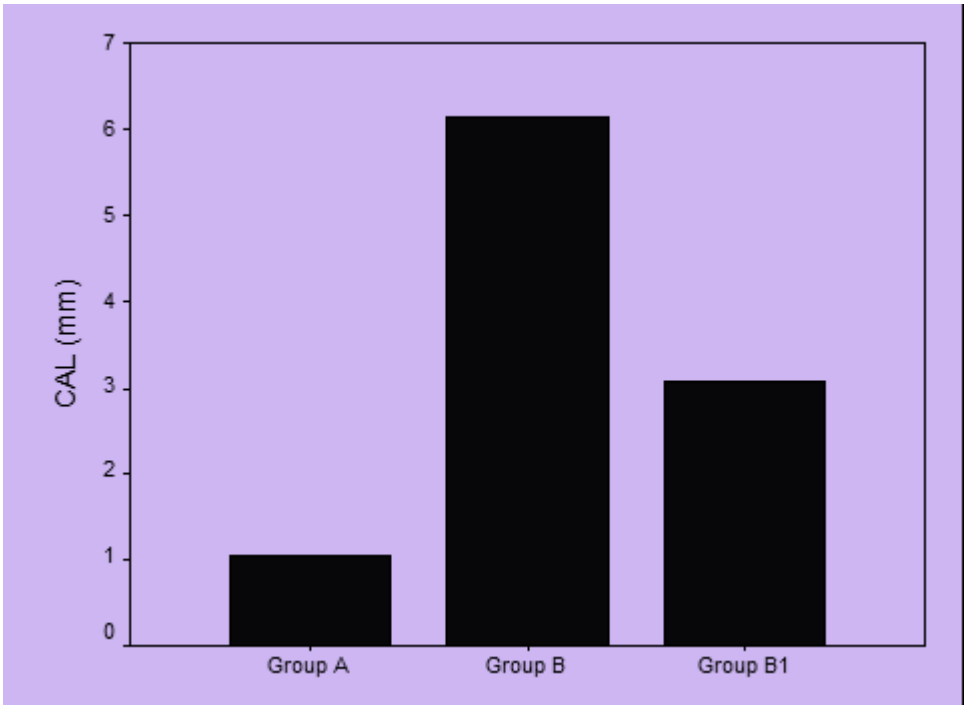
Graph - 3: Comparision Of sites with bleeding on probing in CP Patients before and after NSPT



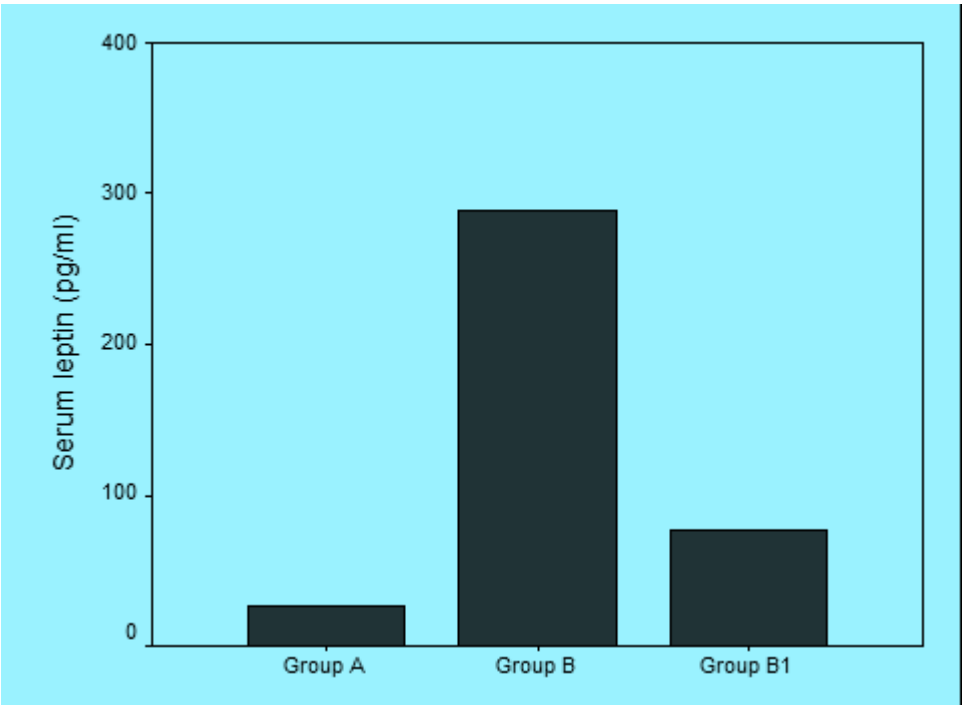
Graph - 4: Comparision Of Probing Depth among healthy and in CP Patients before and after NSPT



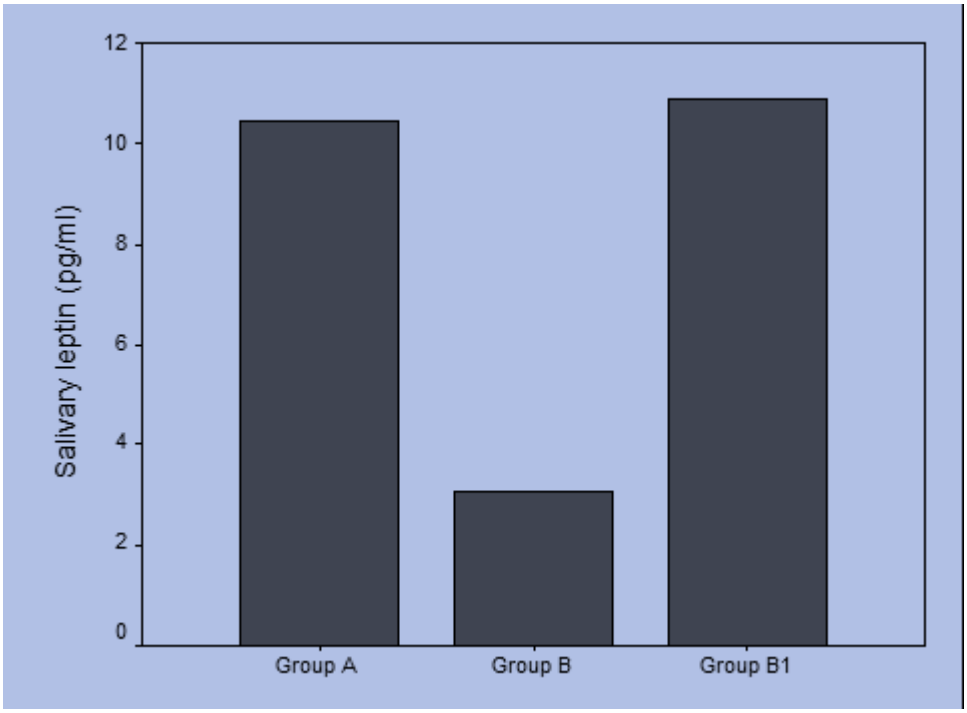
Graph - 5: Comparision Of Clinical Attachment Level among healthy and in CP Patients before and after NSPT



Graph -6: Comparision Of serum leptin levels among healthy and in CP Patients before and after NSPT



Graph -7: Comparision Of salivary leptin levels among healthy and in CP Patients before and after NSPT



Discussion

Chronic periodontitis which is the second most widespread dental disease[47] is initiated by microbes but progressed by cytokines and other host related factors. The principal finding of the study concentrated on alteration in salivary and serum leptin levelson the grounds that leptin which is a regulator of energy homeostasis,can be considered a link between theneuroendocrine and immune systembecause of its dual nature as a hormoneand a cytokine. It is a part ofthe cytokine network that governs theinflammatory/immune response and hostdefensemechanisms.

This study included participants with normal BMI which is considered as an important criteria because leptin levels are altered in obese individuals, as detected by the following study. Zimmermann in 2013[48] evaluated the local and circulating levels of leptin in obese and normal weight individuals with and without CP. Individuals with normal weight, non periodontitis group showed lowest levels of serum leptin. Among the obese group, patients without periodontitis reported leptin concentrations of 479.6 – 253.6 whereas patients with chronic periodontitis showed leptin concentrations of 426.8 – 280.9. Their study implied the dominating role of obesity in the circulating levels of leptin than periodontitis. For this reason, all healthy individuals and chronic periodontitis patients who have a normal BMI were included in this study.

Obesity not only affects the concentrations of leptin but also it perturbs the action of leptin. Ducy et al. conducted a study in obese mice to assess the role of leptin in bone remodeling. Leptin expression could not be detected in whole bone samples even after a long exposure, providing an indirect argument against a paracrine regulation of osteoblast function by leptin. The lack of leptin expression in osteoblasts rules out an autocrine mechanism. Osteoblast cultured from mice underwent long term leptin treatment on extracellular matrix synthesis and bone matrix mineralization but observed no difference in

collagen synthesis or formation of mineralization nodules between control and leptin-treated cultures. In contrast, administration of small doses of leptin in the third ventricle of mice, leads to bone remodeling.This latter result signified that osteoblastic function is affected by leptin binding to its hypothalamic receptor and unveils the central nature of bone remodeling regulation. They related their study to humans and conveyed that obese individuals, in whom the serum leptin levels are highly resistant to the biologic effects of leptin. It is because of this resistance, transportation of leptin to the central nervous system is impaired in obese individuals.[49]

Apart from obesity, smokers, pregnant females and aggressive periodontitis were excluded from the study as a result of the following proven studies. Bozkurt et al [50] in 2006 evaluated influence of smoking on GCF leptin concentrations in patients with chronic periodontitis. They reported that GCF leptin levels were significantly lower in smokers than non-smokers. It seems that smoking may interrupt the mechanism regulating leptin levels. In order to comprehend the possible underlying mechanisms on regulation of leptin, Nagayasu S et al., conducted a study to examine the effects of smoking and inflammation on leptin regulation at cellular levels by gene expression analyses. They noticed that nicotine markedly suppressed the adipocyte expression of leptin gene in the presence of macrophages. Additionally, the observed suppressive effects of nicotin could also be mediated by catecholamines but there was no direct suppressive action on adipocytes.[51] In order to avoid leptin derived from smokers biasing the estimation of leptin concentration, smokers were excluded from the study.

Pregnant women had been excluded from the study because it has been demonstrated that they exhibit higher serum leptin levels in pregnant women than in puerperal women which may be ascribable to an excessive production of leptin from maternal adipose tissue

that is stimulated by high concentrations of serum estradiol and leptin production from the placenta.[52]

Shi D et al observed that the plasma leptin level is higher in patients with Aggressive Periodontitis than chronic periodontitis with comparable Probing Depth. Leptin exhibited a positive association with systemic inflammatory markers including WBC and neutrophil counts, IL-1and IL-6 in patients with Aggressive Periodontitis. These results imply that leptin shows an interdependence between local infection and systemic health.[53] Hence, patients with Aggressive Periodontitis were exempted from the study.

In the present study, serum leptin levels are elevated in chronic periodontitis(288.263±64.7439 pg/ml) than healthy individuals (26.412±8.6102 pg/ml), in the same way as other cytokines. These findings corroborate with the studies done by Shimada et al,[19] Sete et al,[54] Purwar et al, [55] Mendoza et al,[56] Karthikeyan et al,[57] Kanoriya et al,[58] Gonclaves et al[59] and Cosidine et al.[60]On the contrary,Mendoza-Azpur G, observed lowest leptin levels in normal weight subjects with CP instead of normal weight subjects without CP.[56]

The etiology for its rise of serum levels in chronic periodontitis was explained by the following studies. Kim SJ et al. found that Leptin potentiates the production of TNF-alpha induced by Prevotella intermedia lipopolysaccharide in monocyte-derived macrophages. This could account for high levels of leptin observed in the serum of patients with periodontitis in this study and other investigations.[61]Williams RCet al.studied whether leptin has a solitary or synergistic action with inflammatory mediators in transforming the gene expression involved in ECM remodelling of Human Gingival Fibroblasts, with a special attention on the collagenase MMP-1, and the stromelysin MMP-3. They found that leptin enhanced the secretion of MMP-1 and MMP-3 in a dose-dependent manner and acts

synergistically with IL-1 to up-regulate MMP-1, MMP-3, MMP-8 and MMP-12 expression by Human Gingival Fibroblasts, which was regulated by Mitogen Activated Protein Kinase and Signal Transducer and Activator of Transcription 3 (MAPK and STAT3) signalling. Their results suggest that degradation of ECM may be deleteriously intensified by hyperleptinaemia.[62] Thus matrix degradation enhanced by hyperleptinaemia could be one of the reasons for progression of periodontitis.

Genco et al. emphasized that the elevated levels of serum leptin stimulate the secretion of endotoxins by adipose tissue triggered by lipopolysaccharides, which further promote the generation of inflammatory cytokines, leading to additional destruction of periodontium.[63]

The variety of enzymes, hormones, antibodies, antimicrobial constituents, and growth factors are incorporated into the oral cavity from the blood through the transcellular and paracellular routes. Hence biologic fluids of the oral cavity, similar to serum, reflects the physiological state of the body, including nutritional, emotional, hormonal and metabolic variations. These properties open doors to a perfect method of exploring health and disease surveillance in clinical settings with just a minute amount of the oral fluid.

Plentiful studies have been carried out to estimate the levels of leptin in GCF. Johnson and Serio[10] in 2001 evaluated leptin concentrations in healthy and diseased gingiva with the use of ELISA. Higher concentrations of leptin is observed in gingival tissues adjacent to a sulcus depth ≤3mm and reduces progressively in inflamed gingiva with periodontal pockets >3mm. These results are similar to the results of the present study, in which the salivary leptin levels are elevated in healthy subjects and reduced in patients with periodontitis. They suggested that the expansion of the vascular network caused by vascular endothelial growth factor during inflammatory conditions enhance the rate of removal of leptin levels

from the diseased gingiva thereby elevating the serum leptin level. They also proposed that high concentrations of gingival leptin play a protective role.

Karthikeyan and Pradeep[64]evaluated GCF leptin levels in periodontally healthy and diseased subjects with normal BMI. The GCF leptin levels were statistically higher in healthy subjects than in those of patients with chronic gingivitis and periodontitis. They speculated that the progressive decline of leptin levels in gingiva during periodontal disease may be due to the lack of adipocytes which will increase the leptin concentration when acted upon by these cytokines.

Similar to the above study, Selvarajan et al.,2015[65]observed a potential protective role for leptin with regard to periodontal health. However, there was a substantial reduction in leptin levels in GCF as the deterioration of periodontal tissues progressed, which leads to deprivation of protective effects of leptin on the gingival tissues due to impairement of leptin receptor expressionas a result of inflammation in vascular endothelial cells. In addition, they also identified that there was no significant difference between GCF levels of males and females. On this ground, gender difference of leptin levels were not considered in the present study.

All the aforementioned studies, [10,64,65] describing about the role of leptin levels in GCF are also applicable to saliva owing to the fact that both saliva and GCF bears a functional equality with serum. Saliva which is a complex biological fluid exhibiting preferential representation of markers,contains a variety of biomolecules, including DNA, mRNA, microRNA, proteins, metabolites and microbiota. The source of leptin in saliva is derived locally from salivary glands, gingiva and reaches via passive diffusion or active transport from the blood. The changes in the salivary concentration of these biomolecules can be used to develop dysregulated biomarkers which helps to identify early oral and systemic

diseases, evaluate disease prognosis and risk, and monitor the response to treatment. Saliva provides an attractive alternative to more invasive, time-consuming, complicated, and expensive diagnostic approaches.[66]

Based on this contemplation, the present study and few other studies by Groschl et al., 2001,[12] Thanakun et al.,2014,[67] Purwar et al.,2015,[55] Khorsand et al., 2016[68] have evaluated salivary leptin levels in chronic periodontitis. All the above studies have proved that salivary leptin levels are significantly lower in chronic periodontitis compared to healthy individuals. These results are consistent with the present study in which the salivary leptin levels of chronic periodontitis patients (3.153± .2058) were significantly lower than healthy individuals (10.450± 3.9180) andshowed a strongly negative correlation to PD and CAL potentiating the protective role ofleptin in oral health

Due to the presence of higher levels of protein concentration in unstimulated whole saliva, it harmonises with systemic clinical conditions more accurately than stimulated saliva.[12] Pertaining to this reason, the present study involved in the collection of unstimulated saliva. Salivary leptin levels of periodontally healthy individuals range from 5.3-17.9 pg/ml which synchronises with the study done by Thanakun 2013[69] using the same ELISA kit (RayBio®, GA, USA). He also found the salivary leptin levels of healthy individuals to be 5.77- 17.09pg/ml.

Thomas et al. assessed the effects of human recombinant leptin on a conditionally immortalized human marrow stromal cell which serves as a common precursor for both osteoblasts and adipocytes. Their data showed that osteoblastic differentiation of marrow progenitors was enhanced by leptin whereas late adipocytic differentiation was inhibited by leptin. In addition to its action on the central nervous system, their study substantiated the role of leptin in bone formation on account of its direct effect on high affinity leptin

receptors on cells in peripheral tissues, thus enhancing the proliferation, differentiation of human primary osteoblasts and prolonging its life span by inhibiting apoptosis.[70] It is quite evident from the above study that higher salivary leptin concentrations in the healthy individuals , protected the host from inflammation and infection and maintained bone levels.

The decrease in salivary leptin concentrations in chronic periodontitis may be due to the increased expression of leptin receptors during periodontal inflammation owing to the cytopathic changes which provoke intensified binding of leptin to these receptors, thereby decreasing the concentration of leptin in saliva.[55]

The preventive effects of leptin on bacterial growth have been reported in the respiratory mucosa of mice conducted by Hsu et al. They found that leptin prevented decrease in the synthesis of mucin in salivary glands even after the activation of lipopolysaccharides.[71] It also appears to have anti-inflammatory effects. Salivary leptin exerts significant physiologic effects on oral keratinocytes. Such an effect has an important role in wound healing in the oral mucosa.[72] The exact mechanism of its protective role has not been ascertained yet. It appears that leptin is used as a substrate during the inflammatory process. However, enormous evidence conceptualizes the protective role of leptin at higher concentration in saliva.

As it is obvious that localized infection resulting in chronic inflammation may lead to coronary heart disease(CHD), Periodontal disease is a risk factor for CHD, independent of other classic risk factors. Though lipopolysaccharide and monocyte-related responses provide a link between periodontitis and cardiovascular disease, leptin also has the ability to promote proinflammatory signaling through cytokines and growth factors, leading to the production of adhesion molecules such as vascular cell adhesion molecule 1, E-selectin, and intracellular cell adhesion molecule 1, thereby adherence of monocytes are enhanced,

which may contribute to endothelial dysfunction, atherosclerosis.[73] There has been a hypothesis stating that increased serum leptin concentrations up to 10,000 pg/mL in inflammatory conditions could be a risk marker for future cardiovascular disease but it has to be confirmed by further studies.

Leira estimated serum leptin levels in patients with Chronic Migraine with and without chronic periodontitis (CP). Chronic migraine patients with CP showed significantly higher leptin concentrations than Chronic Migraine patients without CP. Furthermore, leptin was found to be associated with the presence of migraine, concluding that this biomarker could play a role in the pathogenesis of migraine. Therefore, it could be inferred that CP via leptin may be involved in the process of migraine chronification.[74]Based on the previous studies, it can be emphasized that periodontitis via leptin has an association with other systemic diseases.

Non Surgical Periodontal Therapy (NSPT) is a critical aspect of periodontal treatment. Long term success of the periodontal treatment depends predominantly on maintaining the results achieved with phase I therapy and much less on any surgical procedures. Hence this study has performed NSPT to focus on alterations of serum and salivary leptin levels. All the clinical parameters (PI, GI, GBI, PD, CAL) showed improvement after 3 months of NSPT and also showed a significant reduction in serum leptin levels (288.263±64.743 to 77.330± 7.150 pg/ml)and an increase in salivary leptin levels (3.153± .2058 to 10.897± 3.665 pg/ml).

Correspondingly, in a study done by Purwar et al.,[18] serum leptin levels which was higher in chronic periodontitis patients showed a significant reduction after 12 weeks of NSPT and the converse took place in salivary leptin levels. These results are analogous to the present study.

Shimada et al. evaluated the effectiveness of periodontal treatment on serum leptin, interleukin (IL) 6, and C-reactive protein (CRP). Nonsurgical therapy significantly reduced the serum leptin, IL-6, and CRP levels after one month in accordance with the periodontal clinical parameters[19] which was congruent to the present study.

Meharwade et al [75] conducted a split mouth study to evaluate the effect of nonsurgical periodontal treatment with and without local drug delivery on the gingival crevicular fluid (GCF) leptin levels from ninety sites of 30 nonobese chronic periodontitis patients. Clinical parameters improved after periodontal therapy in all patients. Patients who were treated with SRP+ tetracycline local drug delivery showed more significant reduction in leptin levels after 15 days than in patients treated with SRP but GCF leptin reelevated to almost the pretreatment level after 45 days. Their study stressed on the fact that indoctrinating the patient in a recall pattern and reinforcing the patient to maintain meticulous personal oral hygiene is also essential to amend the leptin levels.

The present study proves that periodontitis upregulate serum leptin and downregulate salivary leptin levels and NSPT along with meticulous maintenance care can restore the leptin levels to health. Thus leptin serves as a potential marker to provide insight beyondthe classic clinical and radiographicfindings of the disease process. .Moreover, though leptin can be a indicative biomarker of periodontal disease progression, it is unlikely that one standalone biomarker will present with sufficiently high sensitivity and specificity level to meet the criteria of a diagnostic tool.

Summary & conclusion

The following major conclusions can be drawn from this study:

(1) Patients with chronic periodontitis exhibited higher levels of serum leptin compared to periodontally healthy individuals

(2) Salivary leptin levels were lower in the chronic periodontitis patients than healthy controls

(3) Serum leptin levels correlated positively with probing depth and clinical attachment level whereas salivary leptin levels correlated negatively.

(3) Non-surgical periodontal therapy amended salivary and serum leptin levels towards health.

As the salivary leptin levels decline with the progression of periodontitis, determination of salivary levels of leptin can be done as a simple and non-invasive alternative technique to determine the susceptibility to chronic periodontitis.

It can be concluded that leptin might play a role as a new biomarker associating periodontitis with systemic diseases and NSPT not only halt the progression of periodontitis alone but also eliminates the risk of developing systemic diseases. Though advanced modalities of periodontal therapy have been developed, NSPT still remains the cornerstone of periodontal treatment and systemic health maintenance.

Bibliography

1. Arnalich F, Lo´pez J, Codoceo R, Jim nez M, Madero R,Montiel C. Relationship of plasma leptin to plasmacytokines and human survivalin sepsis and septicshock. J Infect Dis 1999;180:908-911.

2. AL-Jumaily EF, Zgaer SH. A Review:"Leptin Structure and Mechanism Actions." Bull Env Pharmacol Life Sci. 2014;3(5):185–192.

3. Popovic V, Damjanovic S, Dieguez C, Casanueva FF. Leptin and the Pituitary. Pituitary. 2001 Jan 1;4(1–2):7–14.

4. Bado A, Levasseur S, Attoub S, Kermorgant S, Laigneau J-P, Bortoluzzi M-N, et al. The stomach is a source of leptin. Nature. 1998 Aug 20;394(6695):790–3.

5. Wang J, Liu R, Hawkins M, Barzilai N, Rossetti L. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. Nature. 1998 Jun 18;393(6686):684–8.

6. Magistrelli D, Rosi F. Effect of Technological Treatments on Human-Like Leptin Level in Bovine Milk for Human Consumption. Foods. 2014 Jul 23;3(3):433–42.

7. Masuzaki H, Ogawa Y, Sagawa N, et al. Nonadiposetissue production of leptin: Leptin as a novel placentaderivedhormone in humans. Nat Med 1997;3:1029-1033.

8. Bado A, Levasseur S, Attoub S, et al. The stomach isa source of leptin. Nature 1998;394:790-793.

9. Sanna V, Di Giacomo A, La Cava A, et al. Leptin surgeprecedes onset of autoimmune encephalomyelitis andcorrelates with development of pathogenic T cell responses.J Clin Invest 2003;111:241-250.

10. Johnson RB, Serio FG. Leptin within healthy anddiseased human gingiva. J Periodontol 2001;72:1254-1257.

11. Gordeladze JO, Drevon CA, Syversen U, Reseland JE. Leptin stimulates human osteoblastic cell proliferation, de novo collagen synthesis, and mineralization: Impact on differentiation markers, apoptosis, and osteoclastic signaling. J Cell Biochem. 2002;85(4):825–36.

12. Gröschl M, Rauh M, Wagner R, Neuhuber W, Metzler M, Tamgüney G, et al. Identification of leptin in human saliva. J Clin Endocrinol Metab. 2001;86(11):5234–5239.

13. Zhang Y, Proenca P, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouseobese gene and its human homologue. Nature 1994; 372:425-32.

14. Denver RJ, Bonett RM, Boorse GC. Evolution of Leptin Structure and Function. Neuroendocrinology. 2011;94(1):21–38.

15. Bernotiene E, Palmer G, Gabay C. The role of leptin in innate and adaptive immune responses. Arthritis Res Ther. 2006;85:217.

16. Fernández-Riejos P, Najib S, Santos-Alvarez J, Martín-Romero C, Pérez-Pérez A, González-Yanes C, et al. Role of Leptin in the Activation of Immune Cells. Mediators Inflamm. 2010;10:1–8.

17. Matarese G, Moschos S, Mantzoros CS. Leptin in Immunology. J Immunol. 2005 Mar 15;174(6):3137–42.

18. Purwar P, Khan MA, Gupta A, Mahdi AA, Pandey S, Singh B, et al. The effects of periodontal therapy on serum and salivary leptin levels in chronic periodontitis patients with normal body mass index. Acta Odontol Scand. 2015 Nov 17;73(8):633–41.

19. 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 Aug;81(8):1118–23.

20. Dias Gonçalves 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 Jan;86(1):53–61.

21. Theodore Kelesidis, Iosif Kelesidis, Sharon Chou, and Christos S. antzoros. Narrative Review: The Role of Leptin in Human Physiology:Emerging Clinical ApplicationsAnn Intern Med. 2010 January 19; 152(2): 93–100

22. Licinio J, Mantzoros C, Negrao AB, Cizza G, Wong ML, Bongiorno PB, et al. Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. Nat Med 2000;3(5):575–9.

23. Elmquist JK, Bjorbaek C, Ahima RS, Flier JS, Saper CB. Distributions of leptin receptor mRNAisoforms in the rat brain. J Comp Neurol 2006;395(4):535–47.

24. Robertson SA, Leinninger GM, Myers MG Jr. Molecular and neural mediators of leptin action. PhysiolBehav 2008;94(5):637–42

25. Roth JD, Roland BL, Cole RL, Trevaskis JL, Weyer C, Koda JE, et al. Leptin responsiveness restoredby amylin agonism in diet-induced obesity: evidence from nonclinical and clinical studies. Proc NatlAcad Sci U S A 2009;105(20):7257–62..

26. De Matteis R, Puxeddu R, Riva A, Cinti S. Intralobular ducts of human major salivary glands contain leptin and its receptor. J Anat. 2010;201(5):363–370.

27. Bohlender J, Rauh M, Zenk J, Groschl M. Differential distribution and expression of leptin and the functional leptin receptor in major salivary glands of humans. J Endocrinol. 2014;178(2):217–223.

28. Lucena S, Capela e Silva F, Lamy E, Fialho L, Neves S. Distribution and expression of leptin in major salivary glands of a hyperleptinemia animal model. Experimental Pathology and Health Sciences2016;8 (1): 15-16.

29. C. Mart´ın-Romero, J. Santos-Alvarez, R. Goberna, and V. S´anchez-Margalet, "Human leptin enhances activation and proliferation of human circulating T lymphocytes," Cellular Immunology,2000; vol. 199, no. 1, pp.15–24.

30 F. Maingrette and G. Renier, "Leptin increases lipoprotein lipase secretion by macrophages: involvement of oxidative stress and protein kinase C," Diabetes, 2003;vol. 52, no. 8, pp. 2121– 2128.

31. Wong, P. F.-Y. Cheung, and C. W.-K. Lam, "Leptinmediated cytokine release and migration of eosinophils: implications for immunopathophysiology of allergic inflammation," European Journal of Immunology, 2007. vol. 37, no. 8, pp. 2337–2348,

32. Claycombe K, King LE, Fraker PJ. A role for leptin in sustaining lymphopoiesis and myelopoiesis. Proc Natl Acad Sci. 2008;105(6):2017–2021.

33. N. Kiguchi, T. Maeda, Y. Kobayashi, Y. Fukazawa, and S. Kishioka, "Leptin enhances CC-chemokine ligand expression in cultured murine macrophage," Biochemical and Biophysical Research Communications,2009; vol. 384, no. 3, pp. 311–315.

34. B. Mattioli, L. Giordani, M. G. Quaranta, and M. Viora, "Leptin exerts an anti-apoptotic effect on human dendritic cells via the PI3K-Akt signaling pathway," FEBS Letters, 2009;vol.583, no. 7, pp. 1102–1106.

35. Karmazyn M, Purdham DM, Rajapurohitam V, Zeidan A. Signallingmechanisms underlying the metabolic and other effects of adipokineson the heart. Cardiov Res 2008;79:279-86.

36. Seufert J, Kieffer TJ, Leech CA, Holz GG, Moritz W, Ricordi C, et al. Leptin suppression of insulin secretion and gene expression in human pancreatic islets: implications for the development of adipogenic diabetes mellitus. J Clin Endocrinol Metab. 1999;84(2):670–676.

37. Swellam M, Hamdy N. Association of nonalcoholic fatty liver disease with a single nucleotide polymorphism on the gene encoding leptin receptor. IUBMB Life. 2012;64(2):180–6.

38. Cojocaru Manole, Cojocaru Im, Siloşi I, Rogoz S. Role of leptin in autoimmune diseases. Maedica. 2013;8(1):68.

39. VanSaun MN. Molecular Pathways: Adiponectin and Leptin Signaling in Cancer. Clin Cancer Res. 2013 Apr 15;19(8):1926–32.

40. Smekal A, Vaclavik J. Adipokines and cardiovascular disease: a comprehensive review. Biomed Pap. 2017 Feb 16:102-104.

41. World Health Organization, Regional Office for the Western Pacific, International Association for the Study of Obesity, International Obesity Task Force. The Asia-Pacific perspective: redefining obesity and its treatment.Lancet 2004; 363: 157–63.

42. American Academy of Periodontology Task Force Report on the Update to the 1999 Classification of Periodontal Diseases and Conditions. J Periodontol. 2015 Jul;86(7):835–8.

43. Dudala SR, Arlappa N. An updated Prasad's socioeconomic status classification for 2013. Int J Res DevHealth 2013;1:26-28.

44. Tursky S, Gilmore ND, Glickman I.Reduced plaque formation by the chlormethyl analogue of Vit.C. J Periodontol. 1970; 41(1):41-3

45. LoeH, silness J.Periodontal disease in Pregnancy. Acta Odontol scand 1963; 21: 533-51.

46. Ainamo, J & Bay I. Problems & proposals for recording gingivitis& plaque. Int Dent J 1975;25; 229-235.

47. Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, et al. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. The lancet. 2012;380(9859):2163–2196.

48. 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 May;84(5):624–33.

49. Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, et al. Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. Cell. 2000;100(2):197–207.

50. Bozkurt FY, Yetkin Ay Z, Sütçü R, Delibaş N, Demirel R. Gingival Crevicular Fluid Leptin Levels in Periodontitis Patients With Long-Term and Heavy Smoking. J Periodontol. 2006 Apr;77(4):634–40.

51. Nagayasu S, Suzuki S, Yamashita A, Taniguchi A, Fukushima M, Nakai Y, et al. Smoking and adipose tissue inflammation suppress leptin expression in Japanese obese males: potential mechanism of resistance to weight loss among Japanese obese smokers. Tob Induc Dis. 2012;10(1):3.

52. Mantzoros CS, Moschos SJ. Leptin: in search of role(s) in human physiology and pathophysiology. Clin Endocrinol (Oxf). 1998;49:551-67.

53. Shi D, Liu Y-Y, Li W, Zhang X, Sun X-J, Xu L, et al. Association between Plasma Leptin Level and Systemic Inflammatory Markers in Patients with Aggressive Periodontitis. Chin Med J (Engl). 2015 Feb 20;128(4):528–32.

54. Sete MRC, Lira Júnior R, Fischer RG, Figueredo CM da S, UERJ - Rio de Janeiro State University, Brazil. Serum Adipokine Levels and their Relationship with Fatty Acids in Patients with Chronic Periodontitis. Braz Dent J. 2015 Apr;26(2):169–74.

55. 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 Apr;86(4):588–94.

56. Mendoza-Azpur G, Castro C, Peña L, Guerrero M-E, 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 Jul 1;7(3):e380–6.

57. Karthikeyan BV, Pradeep AR. Gingival crevicular fluid and serum leptin: their relationship to periodontal health and disease. J Clin Periodontol. 2007 Jun;34(6):467–72.

58. Kanoriya D, Pradeep AR, Mallika A, Singhal S, Garg V. Correlation of crevicular fluid and serum levels of retinol-binding protein 4 and leptin in chronic periodontitis and obesity. Clin Oral Investig. 2017 Sep;21(7):2319–25.

59. Gonçalves TED, Zimmermann GS, Figueiredo LC, Souza M de 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 May;42(5):431–9.

60. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med. 1996;334(5):292–295.

61. Kim SJ. Leptin potentiates Prevotella intermedia lipopolysaccharideinduced production of TNF-alpha inmonocyte-derived macrophages. J Periodontal ImplantSci 2010;40:119-124.

62. Williams RC, Skelton AJ, Todryk SM, Rowan AD, Preshaw PM, Taylor JJ. Leptin and Pro-Inflammatory Stimuli Synergistically Upregulate MMP-1 and MMP-3 Secretion in Human Gingival Fibroblasts. PLoS One 2016 Feb 1;11(2): 68-72.

63. 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(11):2075–2084.

64. Karthikeyan BV, Pradeep AR. Leptin levels in gingival crevicular fluid in periodontal health and disease. J Periodontal Res. 2007 Aug;42(4):300–4.

65. 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 JCDR. 2015 May;9(5):48-53.

66. Pfaffe T, Cooper-White J, Beyerlein P, Kostner K, Punyadeera C. Diagnostic Potential of Saliva: Current State and Future Applications. Clin Chem. 2011 May 1;57(5):675–87.

67. Thanakun S, Watanabe H, Thaweboon S, Izumi Y. Comparison of salivary and plasma adiponectin and leptin in patients with metabolic syndrome. Diabetol Metab Syndr. 2014 Feb 14;6:19.

68. 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 Iran. 2016;13(1):1.

69. Thanakun S, Watanabe H, Thaweboon S, Izumi Y. An effective technique for the processing of saliva for the analysis of leptin and adiponectin. Peptides. 2013 Sep;47:60–5.

70. Thomas T, Gori F, Khosla S, Jensen MD, Burguera B,Riggs BL. Leptin acts on human marrow stromal cells toenhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. Endocrinology 1999;140:1630-1638..

71. Hsu A, Aronoff DM, Phipps J, Goel D, Mancuso P. Leptin improves pulmonary bacterial clearance and survival in ob/ob mice during pneumococcal pneumonia: Leptin restores pulmonary host defence. Clin Exp Immunol. 2007 Sep 5;150(2):332–9.

72. Umeki H, Tokuyama R, Ide S, Okubo M, Tadokoro S, Tezuka M, et al. Leptin Promotes Wound Healing in the Oral Mucosa. PLoS One.2014 Jul 17;73-76.

73. Gundala R, Chava VK, Ramalingam K. Association of Leptin in Periodontitis and Acute Myocardial Infarction. J Periodontol. 2014 Jul;85(7):917–24.

74. 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 Sep 17:922-26

75. 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 Jun;44(3):118–25.

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INSTITUTIONAL ETHICS COMMITTEE
VIVEKANANDHA DENTAL COLLEGE FOR WOMEN

SPONSORED BY : ANGAMMAL EDUCATIONAL TRUST

Ethics Committee Registration No. ECR/784/Inv/TN/2015 issued under Rule 122 DD of the Drugs & Cometics Rule 1945.

Dr. J. Baby John	**Chair Person**	**Dr. (Capt.) S. Gokulanathan**	**Member Secretary**
Mr. K. Jayaraman	Social Scientist	Mr. A. Thirumoorthy	Legal Consultant
Dr. R. Jagan Mohan	Clinician	Dr. N. Meenakshiammal	Medical Scientist
Dr. B.T. Suresh	Scientific Member	Dr. R. Natarajan	Scientific Member
Dr. Sachu Philip	Scientific Member	Mr. Kamaraj	Lay Person

No: VDCW/IEC/04/2015

Date: 14.12.2015

TO WHOMSOEVER IT MAY CONCERN

Principal Investigator: Dr. P.S.Viola Esther

Title: Evaluation of salivary and serum leptin concentration in periodontally healthy and chronic periodontitisindividuals before and after non-surgical periodontal therapy.

Institutional ethics committee thank you for your submission for approval of above proposal .It has been taken for discussion in the meeting held on 04 .12.15.The committee approves the project and it has no objection on the study being carried out in Vivekanandha Dental College For Women.

You are requested to submit the final report on completion of project. Any case of adverse reaction should be informed to the institutional ethics committee and action will be taken thereafter.

CHAIRMAN
INSTITUTIONAL ETHICS COMMITTEE
VIVEKANANDHA
DENTAL COLLEGE FOR WOMEN
Elayampalayam-637 205.
Tiruchengode (Tk) Namakkal (Dt),
Tamilnadu.

SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
VIVEKANANDHA
DENTAL COLLEGE FOR WOMEN
Elayampalayam-637 205.
Tiruchengode (Tk) Namakkal (Dt),
Tamilnadu.