A COMPARATIVE STUDY ON SALIVARY AND SERUM LEVELS OF VISFATIN IN PERIODONTALLY HEALTHY INDIVIDUALS AND CHRONIC PERIODONTITIS PATIENTS BEFORE AND AFTER NON-SURGICAL PERIODONTAL THERAPY

Dissertation submitted to

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

In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH II

PERIODONTICS

2016 – 2019

THE TAMILNADU Dr MGR MEDICAL UNIVERSITY, CHENNAI

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation work titled "**A COMPARATIVE STUDY ON SALIVARY AND SERUM LEVELS OF VISFATIN IN PERIODONTALLY HEALTHY INDIVIDUALS AND CHRONIC PERIODONTITIS PATIENTS BEFORE AND AFTER NON-SURGICAL PERIODONTAL THERAPY**" is a bonafide and genuine research work carried out by me under the guidance of **Dr.S.Elanchezhiyan M.D.S.**, Head of the Department, Department of Periodontics, Vivekanandha Dental College for Women, Tiruchengode.

Dr. D.Surya

Post Graduate Student

Department of Periodontics,

Vivekanandha Dental College for Women,

Elayampalayam,

Tiruchengode.

THE TAMILNADU Dr MGR MEDICAL UNIVERSITY, CHENNAI.

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

This is to certify that **DR. D. SURYA,** Post graduate student in the Department of Periodontics, Vivekanandha Dental College for Women, Elayampalayam, Tiruchengode, has done this dissertation titled **"A COMPARATIVE STUDY ON SALIVARY AND SERUM LEVELS OF VISFATIN IN PERIODONTALLY HEALTHY INDIVIDUALS AND CHRONIC PERIODONTITIS PATIENTS BEFORE AND AFTER NON-SURGICAL PERIODONTAL THERAPY "** under our guidance and supervision during her post graduate study period 2016-2019.

This dissertation is submitted to **THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY** in partial fulfillment of the degree of **MASTER OF DENTAL SURGERY, BRANCH II- Periodontics.**

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

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

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

Dr. N. BALAN M.D.S.,
Principal and Head,
Department of Oral Medicine and Radiology,
Vivekanandha Dental College for Women,
Elayampalayam,
Tiruchengode.

TITLE OF DISSERTATION	A COMPARATIVE STUDY ON SALIVARY AND SERUM LEVELS OF VISFATIN IN PERIODONTALLY HEALTHY INDIVIDUALS AND CHRONIC PERIODONTITIS PATIENTS BEFORE AND AFTER NON-SURGICAL PERIODONTAL THERAPY
PLACE OF STUDY	Elayampalayam, Tiruchengode, Namakkal district.
DURATION OF THE COURSE	3 years (2016-2019)
NAME OF THE GUIDE	**DR.S.ELANCHEZHIYAN M.D.S.**
HEAD OF THE DEPARTMENT	**DR.S.ELANCHEZHIYAN M.D.S.**

I hereby declare that no part of the dissertation will be utilized for gaining financial assistance for research or other promotions without obtaining prior permission of the Principal, Vivekanandha Dental College for Women,Tiruchengode. In addition, I declare that no part of this work will be published either in print or electronic form without the permission of the guide who has been actively involved in the dissertation. The author has the right to reserve publishing of work solely with prior permission of the principal, Vivekanandha Dental College for Women, Tiruchengode.

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

Guide &Head of the Department

Signature of the Candidate

THE TAMILNADU DR.MGR MEDICAL UNIVERSITY, CHENNAI

PLAGIARISM CERTIFICATE

This is to certify that this dissertation work titled "**A COMPARATIVE STUDY ON SALIVARY AND SERUM LEVELS OF VISFATIN IN PERIODONTALLY HEALTHY INDIVIDUALS AND CHRONIC PERIODONTITIS PATIENTS BEFORE AND AFTER NON-SURGICAL PERIODONTAL THERAPY**" of the candidate **DR.D.SURYA,** with registration number **241613503** 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 upload thesis file contains from introduction to conclusion pages and results shows 5% of plagiarism in the dissertation.

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

Guide & Supervisor Sign with Seal.

URKUND

Urkund Analysis Result

Analysed Document:	Thesis Final urkund.pdf (D46547062)
Submitted:	1/8/2019 3:04:00 PM
Submitted By:	drsuryajp@gmail.com
Significance:	5 %

Sources included in the report:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4417487/
https://www.hindawi.com/journals/bri/2018/7463796/
https://link.springer.com/article/10.1007/s00784-014-1308-0
https://www.sciencedirect.com/science/article/abs/pii/S0196978111004785
https://journals.tbzmed.ac.ir/JODDD/Manuscript/JODDD-9-11.pdf
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5858068/
https://www.jstage.jst.go.jp/article/josnusd/58/4/58_16-0116/_pdf

Instances where selected sources appear:

18

ACKNOWLEDGEMENT

*I thank **God Almighty**, from whom I receive everything for accomplishing my desires. He made many impossible things possible in my life.*

*I am much obliged and I would like to extend my heartfelt gratitude to **(Capt.) Dr. S. Gokulnathan B.Sc.**, **M.D.S., Dean**, Vivekanandha Dental College for Women, for permitting me to pursue this study.*

*My sincere thanks to **Dr.N.Balan M.D.S., Principal**, Vivekanandha Dental College for Women, encouraged and guided me to complete this work.*

*With submissive ambition, I aspire to register my gratitude to my respected Head of the Department and guide **Dr.S.Elanchezhiyan M.D.S.,** Department of Periodontics, Vivekanandha Dental College for Women for encouraging me to join post graduation in this department. I felt very much challenged in the beginning of my course as I found it difficult to adapt myself in student life after a long break from my UG. your guidance, support, care and continuous showering of love has motivated me to cross through all the barriers and obstacles and made me stand where I am today. More than a guide and staff you have been a guru to me. I thank you sir to made me join in this college and particularly in this department.*

*I express my special thanks to my Professor **Dr. Rajkumar Daniel M.D.S**. who always supports me with smiling face and encouragement**.** My special and sincere thanks to **Dr. Ramya Nethravathy M.D.S,** Reader and **Dr. Vennila***

M.D.S, *and* ***Dr. Gayathri M.D.S*** *Senior lecturer who guided me with timely advice, meticulous scrutiny and concern in every aspect of my study.*

I would like to extend my love to ***Dr Lekha, Dr. Saranya, Dr. Suganya and Dr. Anathalakshmi.***

My heartfelt gratitude and prayer of my parents ***N.Duraisamy*** *and* ***D.Shanthi*** *and my sister* ***D. Divya*** *And My brother* ***Gowtham****, my mother in law* ***K.Vasanthi*** *and sister in law* ***K.Hemalatha*** *who made possible my tomorrow by sacrificing their yesterday and for their evergreen love and support for which I will be eternally indebted.*

My heartfelt thanks to my husband ***Dr.K.Jayaprakash,*** *for his love and belief in me. I would like to extend my gratitude to my children* ***J.Dhanya*** *and* ***J.Dhanvanth.***

My sincere thanks to my evergreen friend and senior PG ***Dr.T.B.Meenalochani****, for her support right from the days of BDS till now for her non-ending support.*

My since thanks to my co-PGs ***Dr.S.Saranya, Dr.A.S.Abarnasree*** *for their support in the completion of this study.*

My final and heartfelt thanks to my lovable sisters ***Dr.T.Pavithra, Dr.P.S.Viola Esther, Dr.V.Janani Priya, Dr.M.Preethi, Dr.P.Niveditha, Dr. Nirosa, Dr. Subhasini, Dr. Aarthi*** *for their never-ending help.*

CONTENTS

ANNEXURE – I (TABLES)

TABLE NO	TITLE
1	DEMOGRAPHIC CHARACTERISTICS OF STUDY POPULATION
2	COMPARISON OF CLINICAL PARAMETERS BETWEEN HEALTHY AND CHRONIC PERIODONTITIS
3	COMPARISON OF BIOCHEMICAL PARAMETERS BETWEEN HEALTHY AND CHRONIC PERIODONTITIS
4	COMPARISON OF CLINICAL PARAMETERS BEFORE AND AFTER NSPT
5	COMPARISON OF BIOCHEMICAL PARAMETERS BEFORE AND AFTER NSPT
6	CORRELATION OF SERUM AND SALIVARY VISFATIN WITH PD AND CAL

ANNEXURE – II (GRAPHS)

TABLE NO	TITLE
1	COMPARISON OF AGE
2	COMPARISON OF BMI
3	COMPARISON OF PLAQUE INDEX BETWEEN GROUP A, B1 AND B2
4	COMPARISON OF GINGIVAL INDEX BETWEEN GROUP A, B1 AND B2
5	COMPARISON OF BLEEDING ON PROBING (%) BETWEEN GROUP A, B1 AND B2
6	COMPARISON OF PROBING DEPTH BETWEEN GROUP A, B1 AND B2
7	COMPARISON OF CLINICAL ATTACHMENT LOSS BETWEEN GROUP A, B1 AND B2
8	COMPARISON OF SERUM VISFATIN LEVEL BETWEEN GROUP A, B1 AND B2
9	COMPARISON OF SALIVARY VISFATIN LEVEL BETWEEN GROUP A, B1 AND B2
10	GROUP – A - CORRELATION OF SERUM & SALIVARY VISFATIN LEVELS WITH PD & CAL
11	GROUP – B1 - CORRELATION OF SERUM & SALIVARY VISFATIN LEVELS WITH PD & CAL
12	GROUP – B2 - CORRELATION OF SERUM & SALIVARY VISFATIN LEVELS WITH PD & CAL

INTRODUCTION

Introduction

Periodontitis is a multifactorial disease which depends upon periodontal pathogens, the interplay between host activated inflammatory and immunological cascades and even genetic predisposition. This complex process results in the destruction of periodontal connective tissue and alveolar bone breakdown in the periodontium.[1]

Periodontal destruction is mediated by locally produced proinflammatory cytokines in response to bacterial infection.[2] Proinflammatory cytokines, such as IL-6 and TNF-α, are considered to be associated with periodontal inflammation.[3]

Adipose tissue produces a variety of cytokines and inflammatory molecules, commonly referred to as adipo(cyto)kines, which regulate different inflammatory processes and are involved in the path physiology of periodontitis.

Adipose tissue synthesizes many inflammatory factors, including adiponectin, resistin, leptin, and visfatin, as well as cytokines such as tumour necrosis factor.[4]

Visfatin was originally cloned by **Samal et al**. when he studied human pre-B lymphocytes in search of new cytokine-like molecules. They found a 52-kDa secreted molecule named pre-B cell-enhancing factor (PBEF).It was induced by pokeweed mitogen and cycloheximide and it also improved the effect of stem cell factor and IL-7 on pre-B cell colony formation.[5]

In **2005**, **Fukuhara et al**. identified a new adipokine and named it Visfatin to denote that it is expressed at much higher levels in visceral fat than in subcutaneous fat. It turned out to be the same molecule as PBEF.[6]

Visfatin, originally named as pre-B-cell colony enhancing factor (PBEF) and nicotinamide phosphoribosyl transferase(Nampt), is a 52-kDa protein synthesized and secreted from adipose tissues and several cell types, including human peripheral blood monocytes, lymphocytes, macrophages dendritic cells, bone marrow cells, hepatic cells, skeletal muscles, trophoblasts, and fetal membranes.It also inhibits the biosynthesis of nicotinamide adenine dinucleotide. It has a broad spectrum of effects which is exhibited by its potential involvement in a large range of disorders which includes metabolic disorders, inflammatory diseases, septicaemia, myocardial infarction, atherosclerosis, aging, malignancies and neurodegenerative disorders.[6]

The presence of visfatin in a large variety of white blood cells and the tissue-bound macrophage suggests that visfatin plays an important role in the regulation of immune and defence functions.[7]It also acts as a biomarker, growth factor,enzyme and also induces destructive cytokines (IL-1b, TNF-α, and IL-6) in response to infection and inflammation.[8] And conversely increased proinflammatory cytokines such as IL-6, IL-Iβ and TNF-α can significantly increase visfatin expression.[9]

Visfatin is considered an inflammatory adipokine that is available in inflammatory cells and inflammatory conditions. For example, the expression of visfatin increases in acute and chronic inflammatory conditions like rheumatoid arthritis, sepsis, acute lung injury, inflammation, inflammatory bowel disease and psoriasis and plays an imortant role in the persistence of inflammation through its ability to inhibit apoptosis of neutrophils.[10]

Pradeep at al. (2011)[11] demonstrated that visfatin levels increase progressively in both GCF and serum as periodontal disease progresses. **Mamali et al**. **(2012)**[12] have also suggested that visfatin acts as a biomarker in saliva and also

could be used to detect the periodontal disease activity. Thus visfatin with its multitude of inflammatory functions is an ideal candidate biomarker for investigation in periodontitis..

Non-surgical periodontal therapy is an anti-infective therapy with mechanical and chemotherapeutic approaches to minimize or eliminate the microbial biofilm, which is the primary etiology of gingivitis and periodontitis. Scaling and root planning (SRP)is considered the gold standard of non- surgical periodontal treatment. There have been only very few studies which have evaluated the effect of SRP on GCF and serum visfatin.

In our study we endeavoured to find the serum and salivary concentrations of visfatin in healthy controls and patients with chronic periodontitis and to evaluate the effect of NSPT on Visfatin concentration in both serum and saliva.

AIM AND OBJECTIVES

AIM

To evaluate the effect of non-surgical periodontal therapy on salivary and serum concentrations of visfatin in chronic periodontitis patients.

OBJECTIVES

- To compare the salivary and serum visfatin concentrations between healthy individuals and chronic periodontitis patients.
- To correlate the salivary and serum visfatin levels with clinical parameters before and after non-surgical periodontal therapy.
- To assess whether non-surgical periodontal therapy (NSPT) can alter the salivary and serum visfatin levels in chronic periodontitis patients.

REVIEW OF LITERATURE

ADIPOKINES

Kern et al (2003)[13] studied plasma adiponectin levels and adiponectin mRNA levels in adipose tissue of non-diabetic patients. They found that Plasma adiponectin level was inversely related to levels of TNF-α a pro-inflammatory cytokine. Also adiponectin mRNA levels in adipose tissue was higher in women and in lean patients, was associated with high insulin-sensitivity and decreased TNF-α expression.

Bokarewa et al (2005)[14] evaluated the inflammatory activity of resistin by injecting it intra-articularly into mice joints. They found that resistin has strong pro-inflammatory action. It caused an upregulation of pro-inflammatory cytokines mainly IL-6 and TNF-α and caused arthritis in healthy joints.

Bulcao et al (2006)[15] stated that Adipose tissue is now considered to be the biggest endocrine organ of the human body. It secretes a number of substances known as adipocytokines namely adiponectin, leptin, resistin. They have multiple functions in metabolic profile and immunological mechanisms.

Kiguchi et al (2009)[16] studied the effect of leptin on CC-chemokine ligands (CCLs) in murine macarophages. They found that Leptin enhanced the mRNA expression of CCLs namely CCL-3, CCL-4 and CCL-5 via activation of the JAK2-STAT3 (Janus Kinase 2 – signal transducer and activator of transcription 3) pathway.

SALIVARY ADIPOKINES

Mamali et al (2012)[12] studied the adequacy of saliva as an alternative to serum in determination of adipokines levels. Peptides enter the salivary glands either by active transport mechanism or are expressed and secreted by the salivary glands themselves. They identified all adipokines including visfatin in saliva.

SRP

Tunkel et al (2002)[17] performed a systematic review of 27 articles to determine the efficacy of ultrasonic scalers in comparison to manual hand scalers in the treatment of chronic periodontitis. They concluded that though there was no difference between machine-driven and manual debridement in treatment of chronic periodontitis, the time required was less in ultrasonic/sonic subgingival debridement.

Ryan (2005)[18] stated that SRP is considered the gold standard of non-surgical treatment of periodontitis, with its efficiency is reducing the microbial load, BOP and probing depths and improvements in CAL.

Smiley at al (2015)[19] in their systematic review stated that SRP resulted in 0.5 mm average improvement in CAL. SRP with adjuncts resulted in improvements of CAL by 0.2-0.6 mm over those achieved by SRP alone.

VISFATIN

Fukuhara et al (2005)[6] discovered Visfatin, a newly identified adipocytokine which is present mostly in the visceral fat of humans and its expression is associated with development of obesity. It is a 52 kDa cytokine expressed in lymphocytes and is similar to pre-B cell colony enhancing factor (PBEF). It has insulin mimetic activity.

VISFATIN – SECRETION & SOURCES

Curat et al (2006)[20] did selective isolation of maure adipocytes and macrophages from human visceral WAT (White Adipose Tissue) by CD14 immunoselection and found that macrophages population were increased in obese human visceral WAT and was responsible for the enhanced production of visfatin.

Moschen et al (2007)[8] set out to study the immunological and inflammatory functions of visfatin by studying the effect of visfatin on leucocytes. They

hypothesized that macrophages, dendritic cells and colonic epithelial cells may be consider as sources of visfatin as found by confocal microscopy.

Garten et al (2010)[21] studied HefG2 and primary rat and human adipocytes and found that nicotinamide phosphoribosyltransferase (NAMPT / PBEF / Visfatin) was secreted from hepatocytes in addition to adipocytes. But the NAMPT secreted from hepatocytes were less active.

VISFATIN – FUNCTIONS

Moschen et al (2007)[8] studied visfatin and it role in immune and inflammatory functions. They found that

1. Recombinant visfatin activates human leucocytes and induces cytokine products.
2. Visfatin induces production of IC-1β, TNF-α, IL-6 in CD-14^{+}monocytes.
3. Visfatin causes activation of NF-κβ.
4. Plasma visfatin levels and its MRNA expression is increased in colonic tissue of inflammatory bowel disease patients.
5. Macrophages, Dendritic cells and colonic epithelial cells are additional sources of visfatin.

Luk et al (2008)[22] found that VF/PBEF/NAMPT exerts three district important functions of cellular energetics and innate immunity.

1)As NAMPT, it controls the rate limiting step in the salvage pathway of NAD biosynthesis, thereby regulating cellular NAD levels, NAD dependent enzymes and cellular energetics.

2) PBET is released from a variety of cells, and is elevated in inflammatory diseases like sepsis, ALI, RA, IBD, MI. It induces the release of inflammatory

cytokines like TNF-α, IL-1β, IL-6. Through its capacity to inhibit neutrophil apoptosis, it plays a key role in persistence of inflammation.

3) VF has a number of insulin mimetic effects.

VISFATIN IN INFLAMMATION

Xiao et al (2015)[23] studied the involvement of visfatin in inflammation and apoptosis in wistaria rats. They found that

1. Macrophages levels were highly increased in the spleen samples of visfatin group.

2. Visfatin promoted the expression of CD68 and caspase-3 in rat spleen, whereas in LPS-induced acute inflammation, visfatin inhibited the expression of CD68 and activated caspase-3 in rat spleen.

3. Visfatin plays a dual role in the apoptosis in rat spleen, which is mediated by the mitochondrial pathway wherein it had a proapoptotic effect on normal rat spleen, but it exerted an anti-apoptotic effect during lipopolysaccharide induced lymphocyte apoptosis in rat spleen.

4. Visfatin modulated both the proinflammatory and anti-inflammatory cytokines and in rat spleen, such as IL-10, IL-4, IL-6, TNF-α and IL-1β.

Busso et al (2008)[24] studied visfatin/NAMPT which is the rate limiting enzyme in the salvage pathway of NAD biosynthesis from nicotinamide. They studied mice with collagen-induced arthritis and confirmed increased expression of NAMPT in both serum and arthritic paw of mice. They found that a specific competitive inhibitor of NAMPT reduced arthritis severity with comparable activity to etanercept and decreased pro-inflammatory cytokine secretion in affected joints. This pharmacological inhibition of NAMPT reduced the intracellular concentration of NAD in inflammatory cells and pro-inflammatory cytokine (TNF-alpha) secretion by

inflammatory cells. This mechanism might be how NAMPT links NAD metabolism to inflammatory cytokine secretion by leukocytes, and its inhibition might have therapeutic use in immune-mediated inflammatory disorders.

Kang et al (2013)[25] studied melatonin and its effect on visfatin induced iNOS expressed in macrophages which is an important component in pathological inflammation development. They found that melatonin inhibited visfatin induced iNOS expression and NO production which was closely associated with a reduction in phosphorylated JAK2/STAT3 levels and with the inhibition of p65 translocation into nucleus thus suggesting that melatonin can be used to decrease visfatin-iNOS axis associated disease development.

VISFATIN & SYSTEMIC DISEASES

Ognjanovic et al (2001)[9] found PBEF was expressed in normal fetal membranes and it was increased when amniotic and chorionic infection occurred. They observed that PBEF expression was increased by LPS, IL-1β, TNF- α &IL – 6 and PBEF response was reduced by dexamethasone. They hypothesized that PBEF could have a central role in the mechanism of infection induced preterm birth.

Li et al (2006)[26] studied plasma visfatin and apelin levels in normal impaired glucose tolerance andType-2DM subjects and their relationship between metabolic parameters and resistin concentration. Fasting and 2-hour post glucose plasma visfatin levels were significantly reduced in diabetics. Also fasting plasma visfatin correlated positively with BMI, WHR and fasting plasma resistin, but negative correlation was associated with HbA1C and 2-hour OGTT glucose.

Chen et al (2006)[27] investigated plasma visfatin levels in type-2DM patients since visfatin was found to act as an insulin analog on the insulin receptor.Type-2DM patients had elevated plasma visfatin levels. Statistical analysis revealed visfatin as an

independent association factor for Type-2 DM, even after full adjustment for known biomarkers. It also revealed that waist to hip ratio and visfatin were independently associated.

Sandeep et al (2006)[10] studied the role of visfatin in type 2 DM and obesity and to examine its association with visceral and subcutaneous fat is Asian Indians. They sound that elevated VF levels wave associated with obesity and visceral fat but not with subcutaneous fat. Visfatin levels were also increased in Type-2 DM.

Otero er al (2006)[28] studied the changes in plasma levels of adipokines like visfatin, resistin, leptin and adiponectin in patients with rheumatoid arthritis. They found that plasma levels of visfatin, adiponectin and leptin was markedly increased in RA patients suggesting coordinated roles in the modulation of inflammatory environment in them.

Dahl et al (2007)[29] studied the role of inflammation and its mediators in plaque destabilization. They sound that visfatin levels were increased in foam cell macrophages in plaques and they were found in high concentration in unstable atherosclerotic lesions indicating the possible role of visfatin in destabilization of atherosclerotic plaque.

Fukuhara et al (2008)[6] studied the effects of visfatin in metabolism. They found that visfatin had insulin – mimetic effects in mice and cultured cells with lowering of plasma glucose levels. They also found that visfatin binds to insulin receptor non-competitively with insulin suggesting different sites for binding. Visfatin stimulated glucose uptake by cultured adipocytes and muscle cells and suppressed glucose release by cultured hepatocytes. Mice with visfatin gene mutation were found to have elevated plasma glucose levels.

Ye et al (2008)[30] sound that PBEF / VF levels were significantly elevated in both bronchoalveolar lavage fluid and serum of ALI (Acute lung injury) models and in cytokine or cyclic stretch activated lung microvascular endothelium, which was validated by RT-PCR and immunohistochemistry studies. The presence of specific single nucleotide poly morphisms in visfatin /PBEF gene, decreased gene transcription rate and increased the risk of development of ALI in septic patients.

Gosset et al (2008)[31] studied the role VF on catabolic function of cartilage. They found that VF release from human OA chondrocytes was increased by IL-1β.VF triggered ADAMTS-4 and ADAMTS-5 expression and MMP-3 and MMP-4 synthesis and release and increased PGE_2 in an autocrine and paracrine manner, PGE_2 is one of the main catabolic factors involved in OA&MMP are involved in cartilage degradation.

Liu et al (2009)[32] studied visfatin levels in 250 patients undergoing coronary angiography which included patients with chronic coronary artery disease (CAD), acute coronary syndrome(ACS) and control patients. They found that VF levels were significantly higher in chronic CAD and ACS patients compared with controls. Regression analysis also showed that elevated visfatin levels in CAD and ACS patients was independent of well-known CAD risk factors.

Romacho et al (2009)[33] studied and found that ePBEF / NAMPT / VF, though its intrinsic NAMPT activity, induced iNOS (inducible nitric oxide synthase) in cultured human aortic smooth muscle cells in a concentration dependent manner and it appeared to be a direct contributor of vascular inflammation which is a key feature of atherothrombotic diseases linked to metabolic disorders.

Yun-Hee bae et al (2009)[34] studied and enumerated the role of visfatin in angiogenesis. They indicated that visfatin induced endothelial angiogenesis is composed of two sequential steps.

1) The induction of Erk 1/2 -dependent FGF-2 gene expression by visfatin .

2) FGF- induced angiogenesis.

Carrero at al (2010)[35] conducted a study to test the hypothesis that increased visfatin levels in CKD (chronic kidney disease) patients could be involved in the regulation of appetite and nutrient homeostasis. The found that increasing visfatin levels were associated with increasing anorexia but low serum albumin, cholesterol, triglycerides, and aminoacids (essential and non-essential). They speculated that high visfatin levels in CKD patients might be a counter-regulatory response to central visfatin resistance in uremia.

Park et al (2011)[36] injected visfatin intracerebroventricularly in rats and changes in food intake, body weight, body temperature and locomotor activity were observed. They found that visfatin administration was associated with weight loss, anorexia, hypoactivity and hyperthermia and these sickness responses sickness responses were regulated via the COX and the melanocortin pathway in the brain. Visfatin also increased the levels of pro-inflammatory cytokines, prostaglandin synthesizing enzymes and POMC, an anorexigenic neuropeptide. Indomethacin, a COX inhibitor decreased the effects of visfatin on hyperthermia and hypoactivity, but not anorexia. SHU9119, a melanocortin receptor antagonist, blocked visfatin-induced anorexia but did not affect hyperthermia or hypoactivity.

Yang et al (2013)[37] undertook this experiment to study the role of visfatin in HIF-2α-mediated osteoarthritic cartilage destruction. They found that visfatin gene was a direct target of HIF-2α. In turn visfatin increased mRNA levels and activities of

MMP-3,MMP-12 and MMP-13 in cartilage cells, which was responsible for osteoarthritic cartilage destruction in HIF-2α/ DMM (destabilization of medial meniscus) surgery.

Naz et al(2016)[38] sought to find the correlation between IL-6, a pro inflammatory mediator in atherosclerosis and visfatin which acts as a growth factor for vascular smooth muscle cells in a cross section study on 40proven stable symptomatic CAD patients. They found a strong positive correlation between serum visfatin and IL-6 in male CAD patients suggesting that serum visfatin may be related to pro inflammatory effects in CAD.

Srinivasan et al (2018)[39] sought to study the levels of inflammatory adipokines in saliva as markers for type-2 DM patients. They sound that salivary visfatin concentration was significantly higher in Type- 2DM patients.

Yavuz et al (2018)[40] did a study to analyse whether synovial fluid visfatin levels can be used as a biomarker in TMJ disorders. TMJ synovial fluid was obtained from 60 patients with internal derangement (ID) and TMJ osteoarthritis (OA) and analyzed with ELISA. Visfatin had a positive correlation with TMD pain and ID stage and negative correlation within maximum mouth opening. Its levels were higher in joints with OA changes than in joints with no OA changes.

VISFATIN & PERIODONTAL PATHOLOGY

Nogueira et al (2013)[41] did an in vitro study whether visfatin was produced by PDL (periodontal ligament cells) and whether microbial and biomechanical cells play a role in its synthesis. Cultured PDL cells were exposed to fusobacterium nucleatum and subjected to biomechanical strain. Gene expression of visfatin and toll like receptors (TLR) 2 and 4 were analysed by RT-PCR, visfatin synthesis by ELISA and immunocytochemistry, and NF-κβ nuclear translocation by immunofluorescence.

They found the visfatin was produced by PDL cells and F.nucleatum exposure caused visfatin up regulation in dose and time dependent fastion. Biomechanical loading seemed to have a protective effect against F.nucleatum effect on visfatin expression.

Ozcan et al (2015)[42] tried to determine the possible relationship between visfatin and the presence of periodontopathogens in periodontitis. They studied GCF and plaque samples from healthy individuals and patients with periodontitis. They found that visfatin levels were positively correlated with the presence of porphyromonas gingivalis colonisation in the periodontal pockets. They also found that the presence of EBV in the plaque may also to another factor that causes an increase in visfatin levels.

Ozcan et al (2016)[43] investigated the role of visfatin in periodontitis and tried to understand its mechanism which will enable the development of new therapeutic targets. Tissue biopsy samples from chronic periodontitis patients and healthy individuals were studied for MRNA expression of visfatin, NF-κβ, PI3K, TNF-α, IL-1β using NPCR. They found that increased visfatin was associated with expression of NF-κβ and PI3K, thereby suggesting that visfatin via the NF-κβ, PI3K signaling mechanisms contribute to neutrophil apoptosis inhibition.

VISFATIN IN PERIODONTAL DISEASES

Pradeep et al (2011)[11] studied 3 groups of individuals which included healthy individuals, patients with gingivitis and patients with periodontitis, determined the GCF & serum concentration of visfatin in all 3 groups and evaluated the relationship between the concentration and the disease state. They sound that GCF and serum visfatin concentrations were possibly correlated with severity of periodontal disease with lowest concentration in healthy individuals and highest in periodontitis patients.

Pradeep et al (2012)[44] studied the relationship between serum and GCF concentrations of visfatin in 10 healthy individuals, 10 patients with chronic periodontitis and proper control type 2 DM, and 10 patients with chronic periodontitis but without DM. They sound that serum and GCF visfatin concentrations were higher in patients with type 2 DM and chronic periodontitis than other groups.

Tabari et al (2014)[45] evaluated the salivary concentration of chronic periodontitis patients and its relationship with clinical parameters. They found that salivary visfatin concentration was elevated in chronic periodontitis patients when compared to controls. They also found that there was positive correlation between salivary visfatin concentration and CAL.

Ozcan et al (2014)[46] evaluated the salivary levels of visfatin, chimerin, progranulin and their relationship with periodontal health and disease. Healthy patients, patients with gingivitis and patients with periodontics were enrolled. Clinical periodontal parameters were recorded. ELISA test of adipokines were done. Salivary visfatin levels were elevated in patients with gingivitis and periodontitis in comparison to healthy individuals but there was no difference in between gingivitis and periodontitis groups. Salivary visfatin levels were positively related to PI and GI.

Ghallab et al (2015)[47] sought to investigate the possible role of visfatin in pathogenesis of chronic periodontitis and type 2DM.They studied the protein and mRNA gene expression of visfatin in gingival tissue using ELISA and RT-PCR respectively in 10 healthy individuals, 20 patients with CP and 20 patients with CP and DM. They found that visfatin was significantly elevated in gingival tissues of CP and DM patients when compared to other two groups.

Sarhat et al (2017)[48] compared saliva and serum samples of obese individuals with periodontitis and individuals with normal weight but without

periodontitis and studied their biochemical parameters. They found that serum and salivary visfatin was increased in obese patients with periodontitis. They also found visfatin concentration was positively correlated with IL-6, CRP and resistin levels in both serum and saliva. They concluded that obesity might play a destructive role in the pathogenesis of periodontitis through increased salivary visfatin levels.

Yu et al (2017)[49] investigated the relationship between visfatin and periodontal disease severity in CAD patients and also the biomarkers associated in the periodontal disease. Plasma visfatin concentration clinical, cardiac, dental and lab anatomy evaluations were performed in 496 patients with CAD. They found that plasma visfatin levels were elevated in patients with CAD and periodontal disease, and its increase was associated with CPI (community periodontal index), number of missing teeth, gender and inflammation (hs-CRP, neutrophil to lymphocyte ratio).

VISFATIN IN NSPT & PERIODONTITIS

Raghavendra et al (2012)[50] evaluated the effect of NSPT on GCF and serum concentration of visfatin in healthy controls and on patients with chronic periodontitis. They sound that serum and GCF visfatin concentration was highest in patients with chronic periodontitis which decreased after treatment and lowest in healthy controls. They suggested that visfatin can be considered an inflammatory marker and a potential treatment target in periodontal disease treatment.

Tabari et al (2015)[51] evaluated salivary visfatin concentration in 20 periodontally healthy individuals, 20 patients with moderate to severe chronic periodontitis before and after treatment (NSPT). They found that salivary visfatin concentration were reduced after non-surgical periodontitis. They concluded that salivary visfatin had the potential to be a marker for NSPT response evaluation.

Turer et al (2016)[52] evaluated serum GCF visfatin levels in different stages of periodontal disease and in healthy tissues. They compared these parameters in healthy controls, patients with gingivitis and patients with periodontitis. They found that GCF and serum visfatin levels were highest in periodontitis group and lowest in control group. They also found that visfatin levels were increased with inflammation and decreased following periodontal treatment.

MATERIALS AND METHODS

The study was a prospective case-control experimental study which involved healthy volunteers and patients visiting Department of periodontology, Vivekanandha dental college for women, Elayampalayam performed between June-2017 to June-2018.

The study protocol was devised, submitted to Institutional scientific and ethical committee and their approval was obtained.

The study protocol was explained to volunteers and patients prior to data collection, examination and sample collection and informed consent obtained from them.

A total of 60 subjects with normal Body Mass Index (BMI - 18.5-24.9 kg/m2)[53] were enrolled in the study. Out 0f 60, 30 were healthy volunteers and 30 were patients with chronic periodontitis. Chronic periodontitis patients were diagnosed on the basis of American Academy of Periodontology Task Force Report on the Update to 1999 Classification of Periodontal Diseases and Conditions.[54]

Inclusion criteria:

Group-A – Healthy volunteers:

1. Age – 25-65 years old.
2. Systemically and periodontally healthy individuals
3. Good oral hygiene
4. Plaque index[55] [PI] <1
5. Gingival index[56] [GI] <1
6. Probing depth (PD) <3mm
7. No clinical attachment loss (CAL)

Group – B1 – Chronic periodontitis patients before treatment:

1. Age – 25-65 years

2. Patients with signs of gingival inflammation
3. Presence of bleeding on probing (BOP)
4. Clinical Attachment Loss (CAL)> 4mm
5. Probing depth (PD) – 4-5 mm
6. Radiographic evidence of bone loss.

Group – B2 - Chronic periodontitis patients after treatment:

Group – B1 patients who underwent non-surgical periodontal therapy and re-evaluated after 2 months.

Exclusion criteria:

1. Any periodontal therapy in the past 12 months.
2. Aggressive periodontitis
3. History of smoking/alcohol consumption
4. Any systemic diseases including immunological/inflammatory disorders.
5. Any antimicrobial, anti-inflammatory, immunosuppressive therapy in the past 6 months
6. Pregnancy

Protocol:

A complete case history which included Personal, medical and dental history was obtained from the patients. The clinical parameters were recorded following the clinical samples collection in order to avoid contamination of the samples at the initial and at follow up visits. For all patients, Plaque index (PI)[55] by **Turesky et al 1970**, Gingival index (GI)[56] by **Loe and Silness 1975**, Gingival Bleeding Index (GBI)[57] by **Ainamo and Bay 1975**, probing depth (PD) and clinical attachment loss (CAL) were recorded at first visit and after 2 months.

Turesky et al Plaque Index, 1970:

A Disclosing agent was applied on both the lingual and buccal surfaces and patient was advised to rinse with water. The 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 continuous 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 the mesial facial papilla, facial margin, distal facial papilla and the entire lingual margin of the selected teeth.

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

Ainamo and Bay Gingival Bleeding Index,1975:

A gentle probing of the gingival cervical orifice was performed by utilising the No.23/17 explorer. A positive finding was recorded if bleeding occured within 10 seconds, and then it was expressed as a percentage of the total number of sites examined.

Probing depth and Clinical Attachment Level:

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

Collection of clinical samples:

Clinical samples which included both saliva and blood were collected at baseline and after 2 months during the recall visit. Before the collection of samples the patients were instructed to refrain from eating and drinking (except for water) for two hours. They were positioned comfortably on the dental chair in an upright position.

Collection of saliva

The participants were then instructed to rinse their mouth with distilled water and relax for five minutes. Whole unstimulated saliva of volume (4 ml) was collected by the modified draining method. They were asked to spit every 30 second over a period of 5 min into disposable polypropylene tubes which were fitted with a funnel for ease of collection. Saliva samples thus collected were centrifuged for 5 minutes at 3000 rpm to remove the cell debris and 2 ml of the supernatant fluid was pipetted out and stored at -80^{o}C for further analysis.

Collection of serum

Four millilitres (ml) of blood was collected using 20 gauge needles with a 5 ml syringe from the ante cubital fossa by venipuncture as depicted in figure-2. The blood sample was allowed to clot at room temperature for 1 hour, then serum was extracted by centrifuging the clotted sample at 3000rpm for 5 min. From the extracted serum 2 ml was then transferred to 5ml test tubes and stored at -80^{O}C in a deep cold freezer (as shown in figure-4) till the time of further analysis.

Non-Surgical Periodontal Therapy

In our study Non-surgical periodontal treatment included scaling and root planing (SRP) and initiation of oral hygiene instructions.SRP was performed with the help of Woodpecker ultrasonic scalers and Hu-Friedy hand curettes (Chicago, IL, U.S.A) for chronic periodontitis patients. Scaling was done on the initial day along with the collection of samples. Within 2 weeks root planning was also completed. All patients were instructed to brush twice in a day and dental floss using modified Bass method. During the course of study the patients were refrained from using antimicrobials and mouthwashes. The subjects were regularly followed up once in every 15 days via telephonic contact to ensure whether they followed the oral hygiene instructions properly.

Analysis of Visfatin by ELISA:

The Elabscience Human VF (Visfatin) ELISA kit, USA was used to quantify the serum and salivary visfatin concentrations. The following reagents and materials are used.

Reagents Used:

Item	Specification
Micro ELISA plate	8 wells x 12 strips
Reference standard	2 vials
Concentrated Biotinylated detection Ab (100x)	1 vial – 120 microL
Concentrated HRP conjugate (100x)	1 vial – 120 microL
Reference standard & Sample Diluent	1 vial – 20 mL
Biotinylated Detection Ab diluent	1 vial – 14 mL
HRP Conjugate diluent	1 vial – 14 mL
Concentrated wash buffer (25x)	1 vial – 30 mL
Substrate reagent	1 vial – 10 mL
Stop solution	1 vial – 10 mL
Plate sealer	5 pieces

Other supplies required:

1. Microplate reader with 450nm wavelength filter
2. High-Precision transferpettor, EP tubes and disposable pipette tubes
3. 37^0C Incubator
4. Deionised or distilled water
5. Absorbent paper
6. Loading slot for wash buffer

Reagents preparation:

1. Bring all reagents to room temperature (18-25^{0}C) before use. Preheat the microplate reader 15 minutes before OD preparation.
2. Wash buffer : Dilute 30ml of concentrated wash buffer into 750ml of wash buffer with deionised or distilled water.
3. Standard working solution :
    a. Centrifuge the standard at 10,000 rpm for one minute. Add 1ml of reference standard & sample diluent. Let it stand for 10 minutes and turn it upside down for several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 20ng/ml. Serial dilutions can be made to achieve a dilution gradient of 20,10,5,2.5,1.25,0.63,0.31,0 ng/ml.
4. Biotinylated Detection Ab working solution :
    a. Calculate the required amount before experiment (100µl/well).
    b. Centrifuge the stock tube before use, dilute the concentrated biotinylated detection Ab working solution by biotinylated detection Ab diluent.
5. Concentrated HRP conjugate working solution:
    a. Calculate the required amount before experiment (100µl/well). In actual preparation, we should prepare 100~200µl more. Dilute the conjugated HRP conjugate to working solution by HRP conjugate solution.

Assay procedure :

1. 100µl of standard working solution/samples is added to each well. The plate is covered with sealer. It is incubated for 90 minutes at 37^{0}C.

2. Remove the liquid of each well. Add 100µl of Biotinylated detection Ab working solution to each well. Incubate at 37^0C for 1 hour.
3. Remove the solution from each well. Wash each well with 350µl of wash buffer and dry it. Repeat the wash step 3 times.
4. Add 100µl of HRP conjugate working solution to each well. Cover with sealer and incubate for 30 minutes at 37^0C.
5. Aspirate and wash for 5 times as explained in step-3.
6. Add 90µl of Substrate reagent to each well. Cover with sealer and incubate for 15 minutes at 37^0C.
7. Add 50µl of stop solution to each well.
8. Determine the optical density (OD) value of each well at 450nm immediately.
9. Calculation of results.

Sensitivity

The minimum detectable dose of Human Visfatin was determined to be 0.19 ng/ml.

Specificity

The Visfatin ELISA kit recognises natural and some recombinant Human VF. No significant cross-reactivity or interference between Human VF and analogues were observed.

APPENDIX - 1

PROFORMA
VIVEKANANDHA DENTAL COLLEGE FOR WOMEN
DEPARTMENT OF PERIODONTICS

Title: Evaluation of salivary and serum visfatin concentrations in healthy and chronic periodontitis individuals before and after non-surgical periodontal therapy.

Name: Date:

Age / Sex: OP no.:

Address:

History

Chief Complaints:

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:

BASELINE

Measurement of Periodontal pocket and clinical attachment :

																CA
																PP
DB MB MR DP MP MR	DB MB MR DP MP MR	DB MB MR DP MP MR	DB MB MR DP MP MR	DB MB MR DP MP MR	DB MB MR DP MP MR	DB MB MR DP MP MR	DB MB MR DP MP MR	MB MR DB MP MR DP	MB MR DB MP MR DP	MB MR DB MP MR DP	MB MR DB MP MR DP	MB MR DB MP MR DP	MB MR DB MP MR DP	MB MR DB MP MR DP	MB MR DB MP MR DP	
																PP
																CA
18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28	
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38	
																CA
																PP
DL ML ML DB MB MR	DL ML ML DB MB MR	DL ML ML DB MB MR	DL ML ML DB MB MR	DL ML ML DB MB MR	DL ML ML DB MB MR	DL ML ML DB MB MR	DL ML ML DB MB MR	ML ML DL MB MR DB	ML ML DL MB MR DB	ML ML DL MB MR DB	ML ML DL MB MR DB	ML ML DL MB MR DB	ML ML DL MB MR DB	ML ML DL MB MR DB	ML ML DL MB MR DB	
																PP
																CA

RAGIOGRAPHIC FINDINGS:

DIAGNOSIS:

AFTER 2 MONTHS

Measurement of Periodontal pocket and clinical attachment :

	CA	PP	DB MB MB DP MP MP	PP	CA	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28	CA	PP	
			DB MB MB DP MP MP			48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38			
			DL ML ML DB MB MB																					

VISFATIN ANALYSIS

	BASELINE	AFTER 2 MONTHS
Serum Visfatin level		
Salivary Visfatin level		

விவேகானந்தா பல் மருத்துவக் கல்லூரி
திருச்செங்கோடு - 637205

ஈறுநோய் சிகிச்சைப்பிரிவு
ஒப்புதல் படிவம்

ஆய்வாளர் : து.சூர்யா

பெயர் : ஆண் / பெண்

முகவரி: வயது :

_____ ஆகிய எனக்கு
பரிசோதனை பற்றிய அனைத்து விவரங்களும் தெளிவாக புரியும்படி எடுத்துரைக்கப்பட்டன. என்னுடைய சந்தேகங்கள் அனைத்திற்கும் தெளிவான பதில் அளிக்கப்பட்டது. எனது இரத்த மாதிரி, எச்சில் மாதிரி எடுப்பதற்கும் பரிசோதனைகளுக்கும் முழு மனதோடு சம்மதிக்கின்றேன்.

கையொப்பம் :

தேதி :

INFORMED CONSENT FORM
VIVEKANANDHA DENTAL COLLEGE FOR WOMEN
TIRUCHENGODE

DEPARTMENT OF PERIODONTICS

Research student: Surya. D

Patient Name: **Male/female:**

Address: **Age:**

I have been explained the nature and purpose of the study in which I have been asked to participate. I have been given the opportunity to question about the study and other procedures. I hereby give the consent to be included in this study.

Place:

Date:

Signature of patient

APPENDIX – 2

ARMAMENTARIUM

MATERIALS AND INSTRUMENTS USED FOR CLINICAL STUDY

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

MATERIALS AND INSTRUMENTS USED FOR SAMPLE COLLECTION AND STORAGE

- Saliva collection
    - Polypropylene tube
    - Borosil funnel
- Blood collection
    - Tourniquet
    - 5 ml syringe
- Centrifuge
- Micropipette
- 5 ml Test tube
- Deep freezer

PHOTOGRAPHS

ARMAMENTARIUM FOR NSPT

Figure -1

VISFATIN KIT REAGENTS

Figure - 2

VISFATIN ELISA PLATE

Figure - 3

SAMPLE COLLECTION AND STORAGE

Serum sample collection

Figure – 4

Salivary sample collection

Figure -5

Centrifuge of Samples

Figure – 6

Sample storage in Deep cold freezer

Figure - 7

Preoperative View

Figure - 8

Measurement of PD and CAL by pressure sensitive probe

Figure - 9

Postoperative View

Figure – 10

Visfatin Kit Elisa Plate with Samples

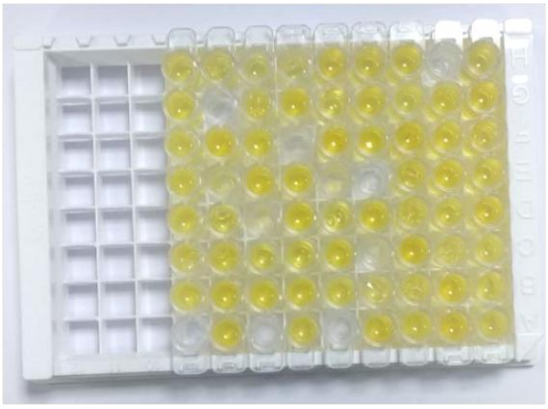


Figure – 11

ELISAWasher and Incubator

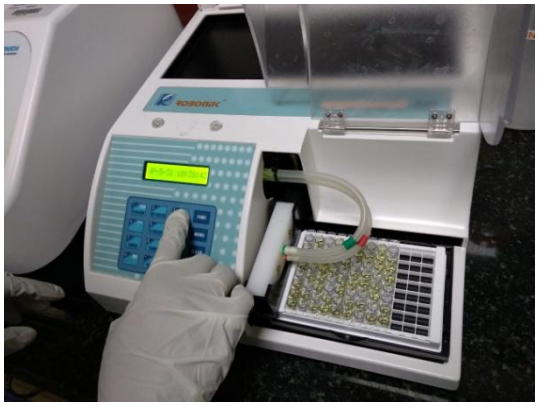


Figure – 12

ELISAReader

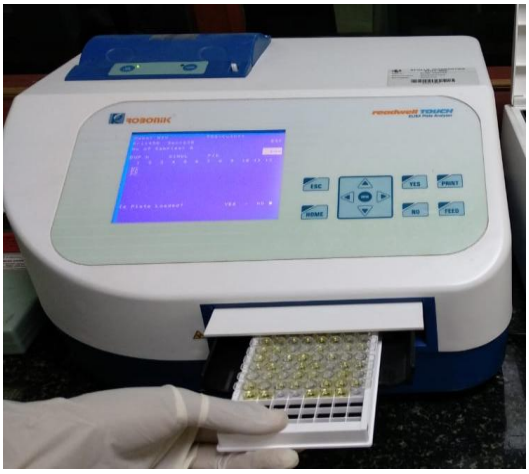


Figure -13

RESULTS

Statistical Analysis

Statistical Package for Social Sciences - SPSS (IBM SPSS Statistics for Windows, Version 23.0, Armonk, NY: IBM Corp. Released 2015) was used to analyse the statistical data. **The Normality tests Kolmogorov-Smirnov** and **Shapiro-Wilks tests** results revealed that the all variables follow Normal distribution. Therefore, to analyse the data Parametric methods were applied. Independent samples t-test were applied to compare mean values between groups. Paired t-test were applied to compare mean values between two time points. To compare proportions between study and control groups Chi-Square test was applied, and if any expected cell frequency was less than five then Fisher's exact test was used. Karl Pearson correlations are calculated to assess the linear relationship between variables.

Sample size calculation

GPower version 3.1.9.2 was used to calculate the sample size. It was calculated that to design a study with 95% power and an alpha error of 0.05 the required sample size was 13. So 30 patients were enrolled for this study, keeping in mind an expected attrition rate of 25%.

Demographic characteristics of the study population

There was no statistically significant difference ($p<0.05\%$) in terms of Age (**Graph -1**), Gender, BMI (**Graph – 2**), Number of teeth and frequency of Brushing in between the two groups group A (healthy individuals) and group B (chronic periodontitis) as determined by T-test and chi-square test as depicted in **Table -1.**

Comparison of clinical parameters between group A (healthy individuals) and group B1 (chronic periodontitis)

Clinical parameters were compared between two groups group A (healthy individuals) and group B1 (chronic periodontitis) using independent t-test. The results revealed that PI (**Graph – 3**), GI (**Graph – 4**), GBI, PD (**Graph – 6**), CAL (**Graph – 7**), % of sites with BOP (**Graph – 5**) of **Group – B** were higher than group – A and the difference was statistically significant as revealed by **Table -2.**

Comparison of salivary and serum visfatin levels between group A (healthy individuals) and group B1 (chronic periodontitis)

Visfatin was found in all salivary and serum samples of both groups. The results of visfatin assay was compared using independent t-test. The mean serum level of visfatin of chronic periodontitis group (38.1± 2.8971) was significantly higher than healthy individuals' group (16.423±2.1148). The mean salivary level of visfatin of chronic periodontitis group (57.86±3.355) was significantly higher than healthy individuals' group (19.943±2.1355). The difference in both serum **(Graph – 8)** and salivary (**Graph – 9**) samples of both groups were statistically significant as shown in **Table – 3**.

Comparision of periodontal parameters in patients with chronic periodontitis before (Group B1) and after (Group B2) NSPT by paired t test

Post – NSPT statistically significant reduction was observed in all clinical parameters of chronic periodontitis patients which included PI,GI, GBI, PD, CAL, % of sites with BOP as shown in **Table – 4**.

Comparison of the biochemical parameters in patients with chronic periodontitis before (Group B1)and after (Group B2) NSPT by paired t test

Serum and salivary levels of chronic periodontitis patients were evaluated 2 months post-NSPT. Serum levels of visfatin were decreased post-treatment (20.323±4.8444) compared to pre-treatment (38.1±2.8971). Salivary levels of visfatin were also decreased post-treatment (19.626 ± 2.6122) compared to pre-treatment (57.860±3.355). The post-treatment decrease in visfatin levels were statistically significant as shown in **Table – 5.**

Pearson's correlation among serum and salivary visfatin levels with Probing Depth (PD) and Clinical attachment loss (CAL)

There was a statistically significant positive correlation between Serum visfatin, PD and CAL in all 3 groups. This was also true for salivary visfatin where in there was a significant positive correlation with PD and CAL. This was demonstrated in **Table – 6.**

LIST OF TABLES

TABLE 1: DEMOGRAPHIC CHARACTERISTICS OF THE STUDY POPULATION

CHARACTERISTICS		GROUP A N=30	GROUP B1 N=30	P VALUE
AGE		46.03 ± 9.031	44.87 ± 8.705	>0.05
GENDER	MALES	50 %	53.3 %	>0.05
	FEMALES	50 %	46.7 %	>0.05
NUMBER OF TEETH		29.37 ± 2.606	29.13 ± 2.515	>0.05
FREQUENCY OF TOOTH BRUSHING	ONCE DAILY	80 %	90 %	>0.05
	TWICE DAILY	20 %	10 %	
BMI		22.803 ± 1.7399	22.657 ± 1.8658	>0.05

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

CLINICAL PARAMETERS	GROUP A N=30	GROUP B1 N=30	MEAN DIFFERENCE	P VALUE
PLAQUE INDEX	0.4057 ± 0.2167	2.9933 ± 0.5938	-2.5876	< 0.001
GINGIVAL INDEX	0.000	2.0633 ± 0.1791	-2.0633	< 0.001
BOP (%)	0.000	99.800 ± 0.6644	-99.800	< 0.001
PD (mm)	2.230 ± 0.5325	5.270 ± 0.8703	-3.0400	< 0.001
CAL (mm)	1.0570 ± 0.4675	6.100 ± 0.8871	-5.0430	< 0.001
PD>4mm (%)	0.000	49.433 ± 10.0533	-49.433	< 0.001
CAL>5mm (%)	0.000	48.013 ± 13.4565	-48.013	< 0.001

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

BIOCHEMICAL PARAMETERS	GROUP A	GROUP B1	MEAN DIFFERENCE	P VALUE
SERUM VISFATIN (ng/ml)	16.423 ± 2.1148	38.100 ± 2.8971	-21.6767	< 0.001
SALIVARY VISFATIN (ng/ml)	19.943 ± 2.1355	57.860 ± 3.3550	-37.9167	< 0.001

TABLE 4: COMPARISION OF CLINICAL PARAMETERS BEFORE AND AFTER NSPT

CLINICAL PARAMETERS	GROUP B1 N=30	GROUP B2 N=30	T	P VALUE
PLAQUE INDEX	2.9933 ± 0.5938	1.6300 ± 0.3958	10.477	< 0.001
GINGIVAL INDEX	2.0633 ± 0.1791	1.1113 ± 0.2029	23.165	< 0.001
BOP (%)	99.800 ± 0.6644	19.800 ± 13.4277	32.553	< 0.001
PD (mm)	5.270 ± 0.8703	2.393 ± 0.6736	27.481	< 0.001
CAL (mm)	6.100 ± 0.8871	3.0933 ± 0.6937	38.600	< 0.001
PD>4mm (%)	49.433 ± 10.0533	29.933 ± 10.3588	31.868	< 0.001
CAL>5mm (%)	48.013 ± 13.4565	29.6333 ± 7.4486	13.808	< 0.001

TABLE 5: COMPARISION OF BIOCHEMICAL PARAMETERS BEFORE AND AFTER NSPT

BIOCHEMICAL PARAMETERS	GROUP B1	GROUP B2	T	P VALUE
SERUM VISFATIN (ng/ml)	38.100 ± 2.8971	20.323 ± 4.8444	17.450	< 0.001
SALIVARY VISFATIN (ng/ml)	57.860 ± 3.3550	19.626±2.6122	16.374	< 0.001

TABLE 6: CORRELATION OF SERUM AND SALIVARY VISFATIN WITH PD AND CAL

			PD	CAL
GROUP A	SERUM VISFATIN (ng/ml)	r	0.981	0.938
		p	< 0.05	< 0.05
	SALIVARY VISFATIN (ng/ml)	r	0.945	0.981
		p	< 0.05	< 0.05
GROUP B1	SERUM VISFATIN (ng/ml)	r	0.874	0.916
		p	< 0.05	< 0.05
	SALIVARY VISFATIN (ng/ml)	r	0.951	0.965
		p	< 0.05	< 0.05
GROUP B2	SERUM VISFATIN (ng/ml)	r	0.901	0.839
		p	< 0.05	< 0.05
	SALIVARY VISFATIN (ng/ml)	r	0.884	0.779
		p	< 0.05	< 0.05

R Value:

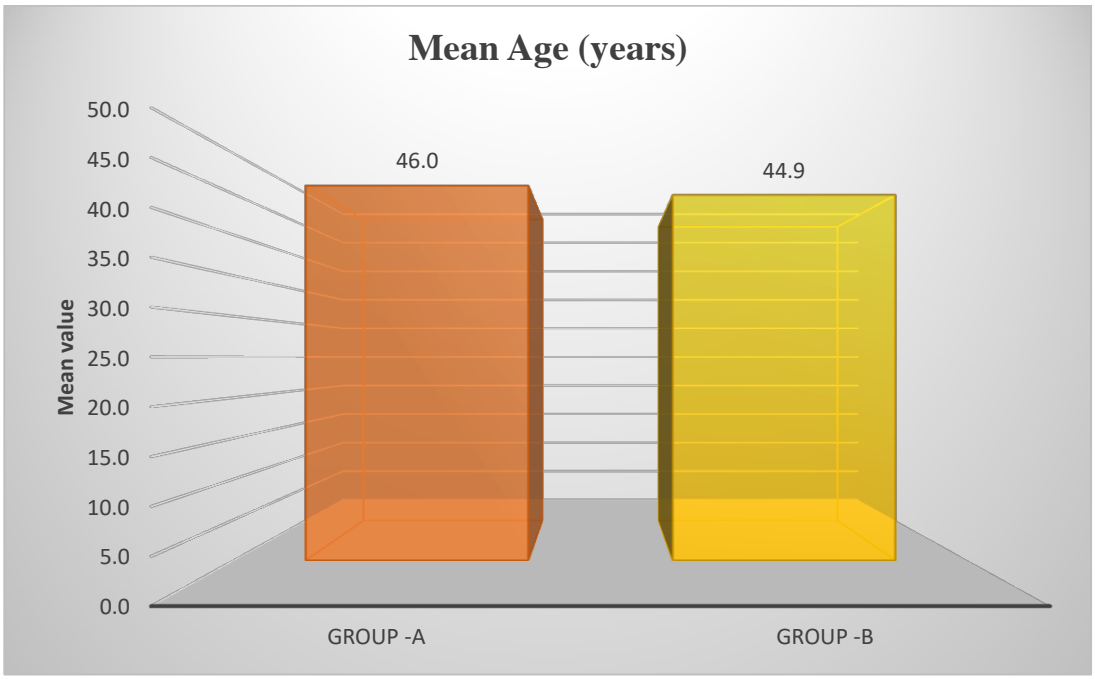
0.1-0.3 - Weak Positive

0.3-0.5 - Moderate positive

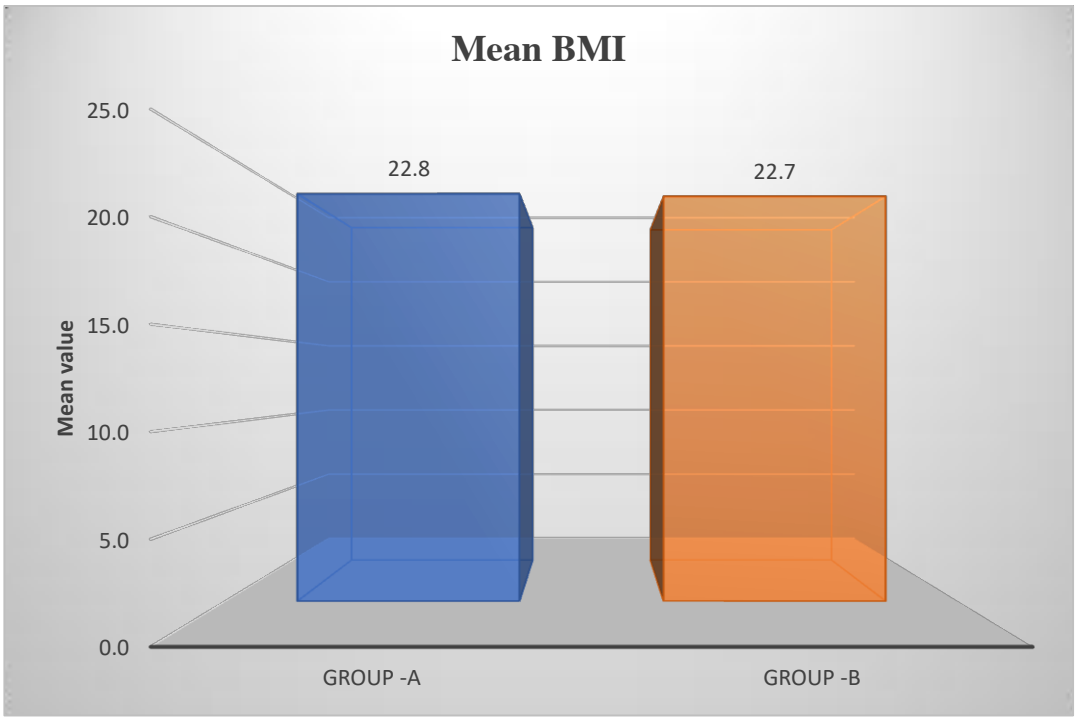
0.5-0.9 - Strong positive

LIST OF GRAPHS

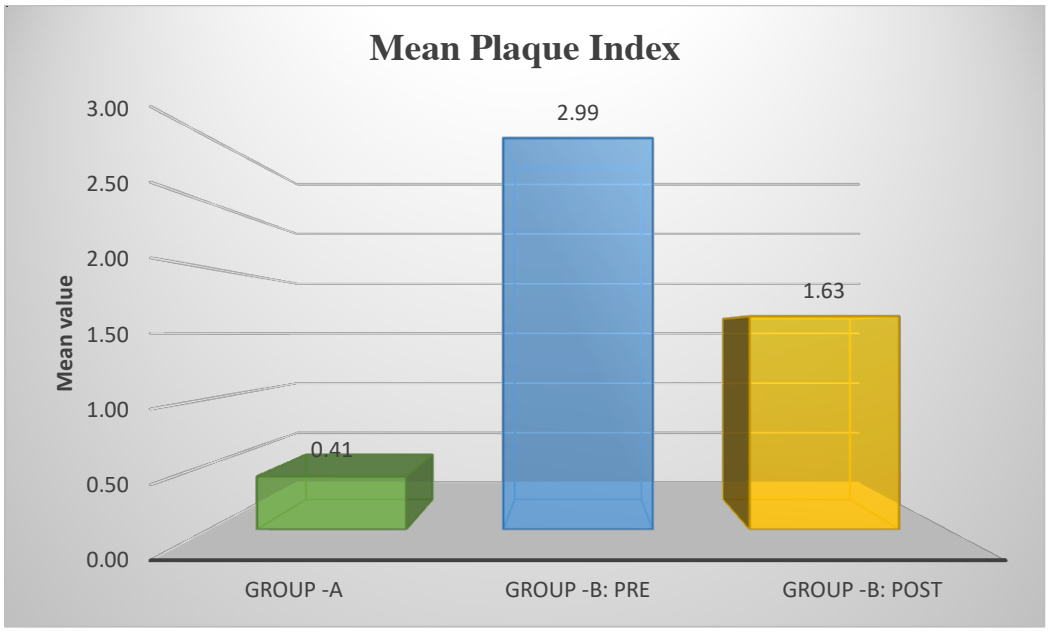
Graph – 1: Comparison of Age



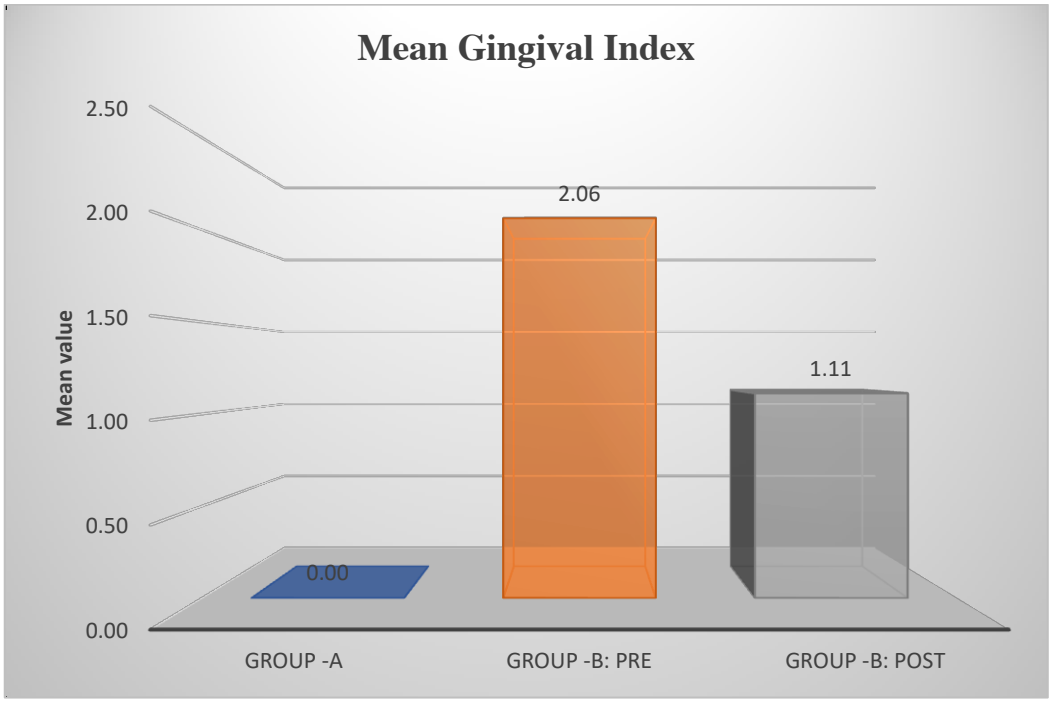
Graph – 2: Comparison of BMI



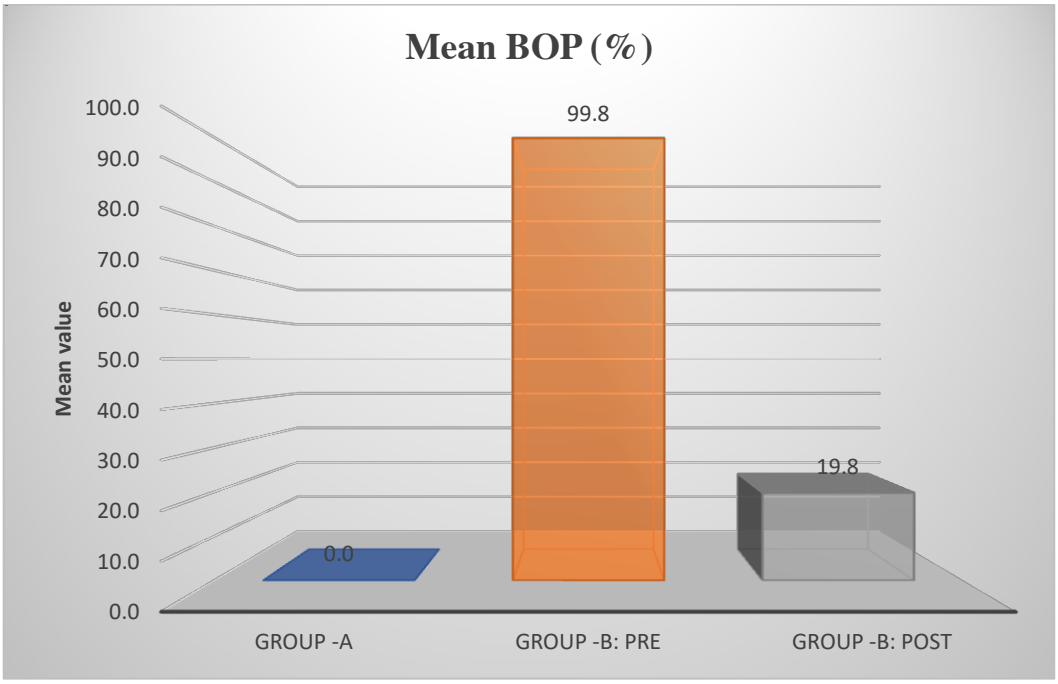
Graph – 3 - Comparison of Plaque index between Group A, B1& B2



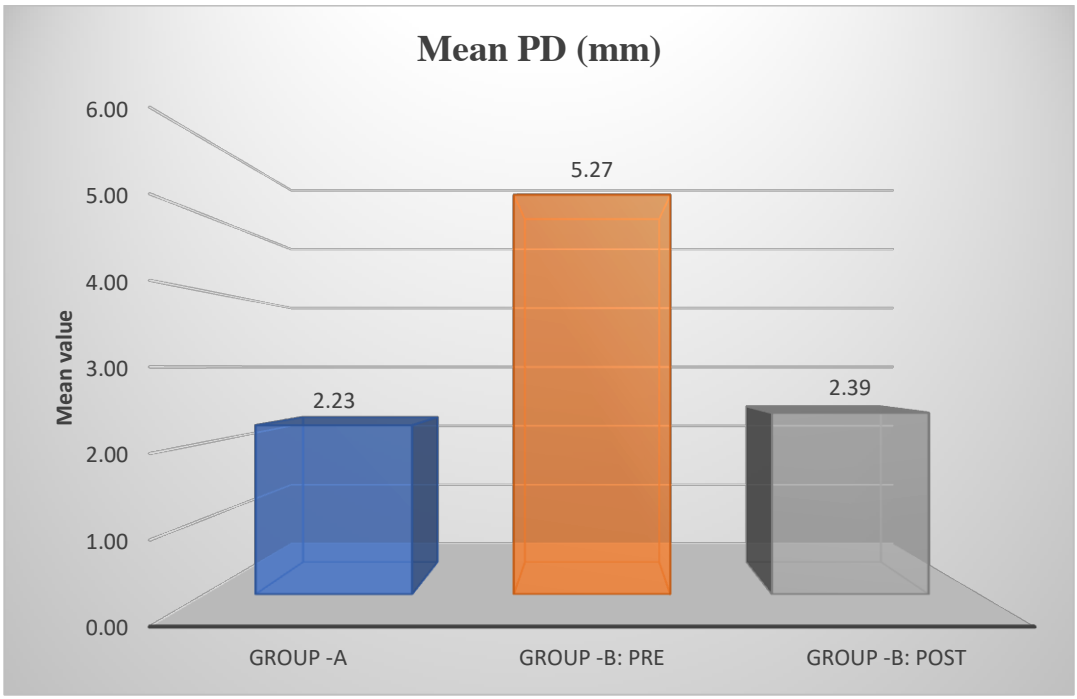
Graph – 4Comparison of Gingival indexbetween Group A, B1& B2



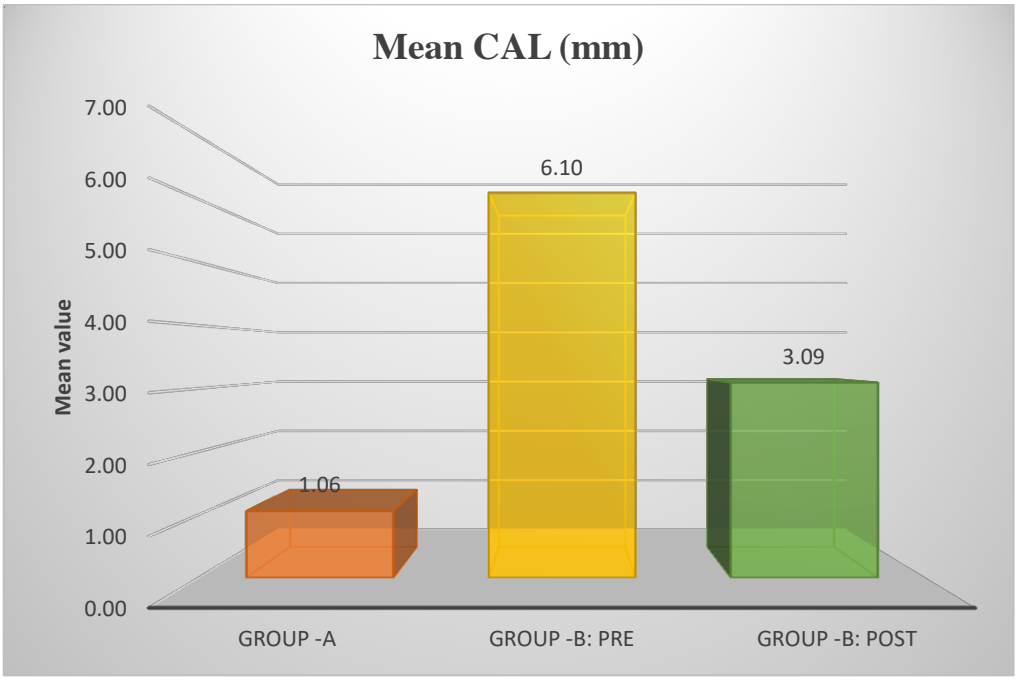
Graph – 5 –Comparison of Bleeding on Probing (%) between Group A, B1& B2



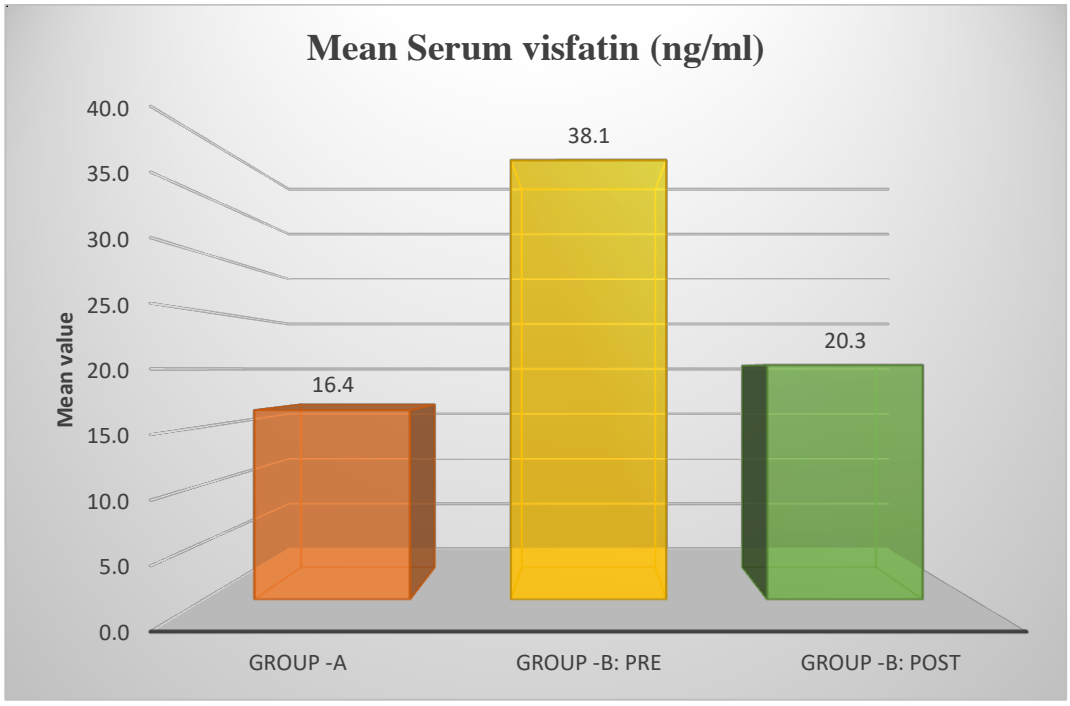
Graph – 6 – Comparison of Probing Depth between Group A, B1& B2



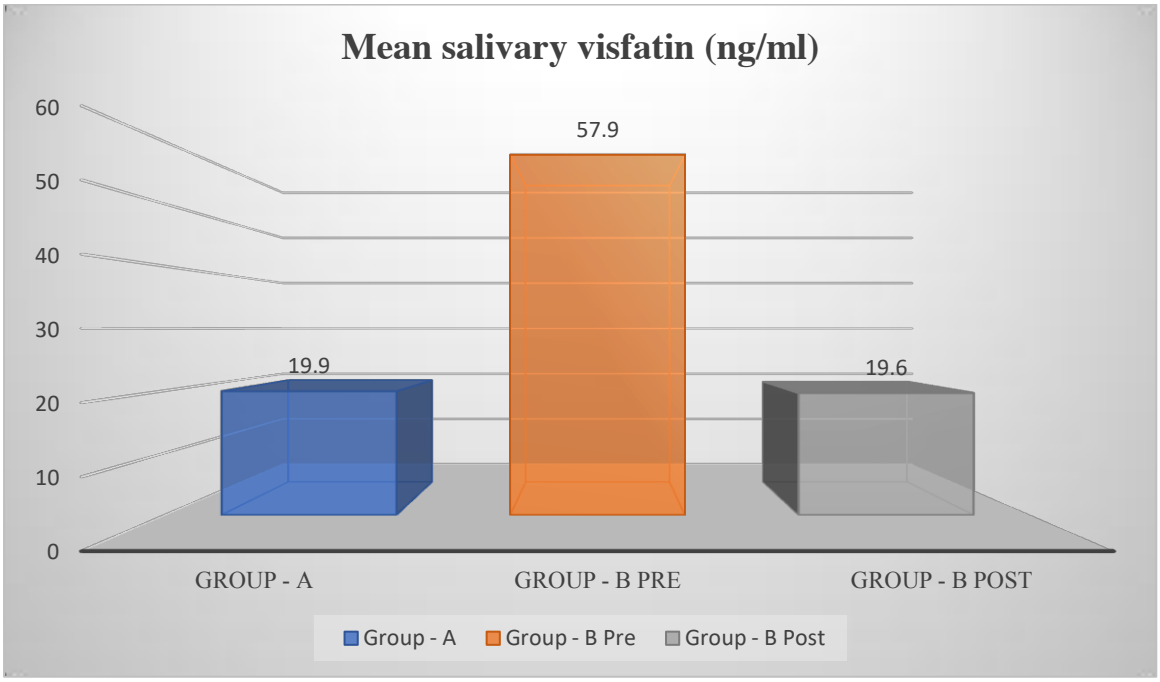
Graph – 7 - Comparison of Clinical Attachment Loss between Group A, B1& B2



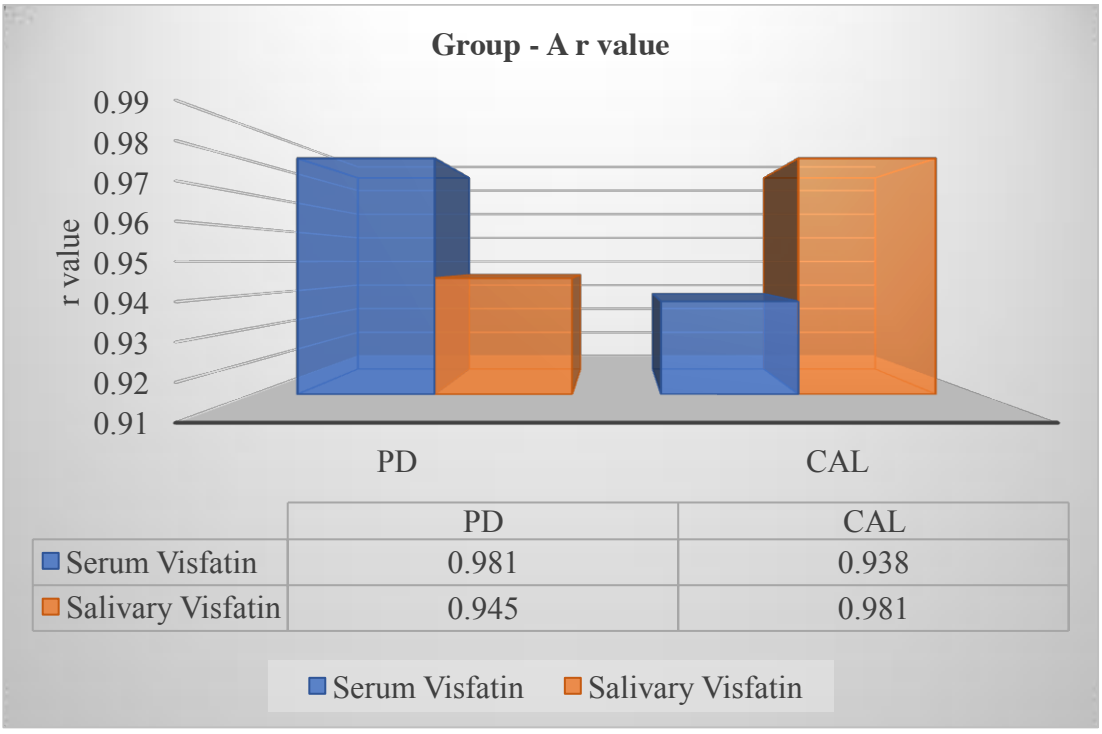
Graph – 8 –Comparison of Serum Visfatin levels between Group A, B1& B2



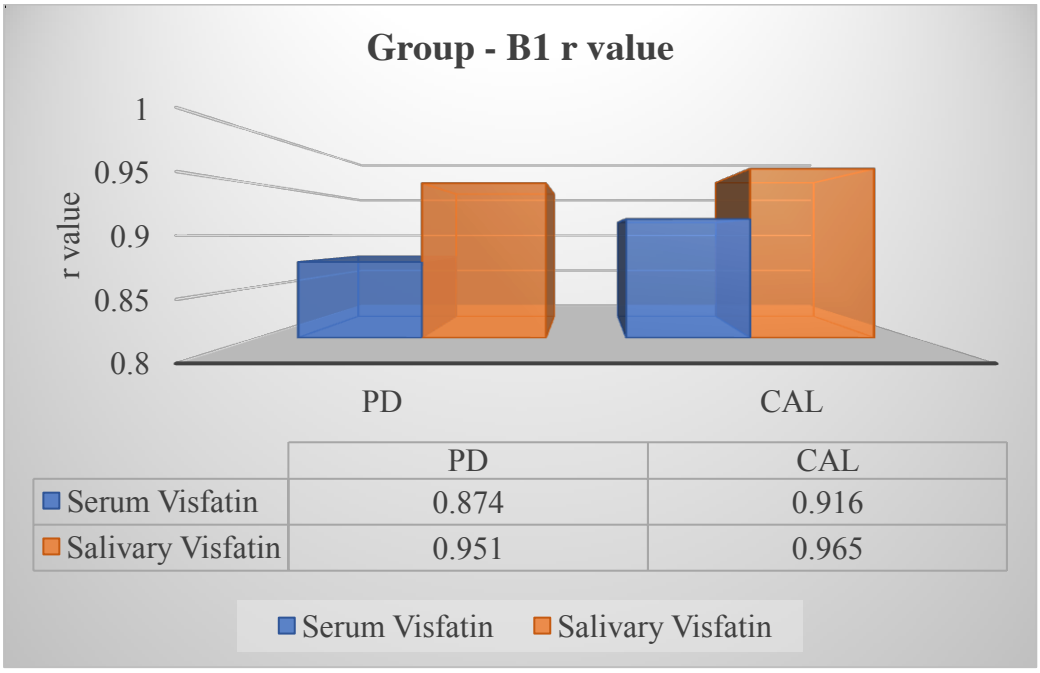
Graph – 9 - Comparison of Salivary Visfatin levels between Group A, B1& B2



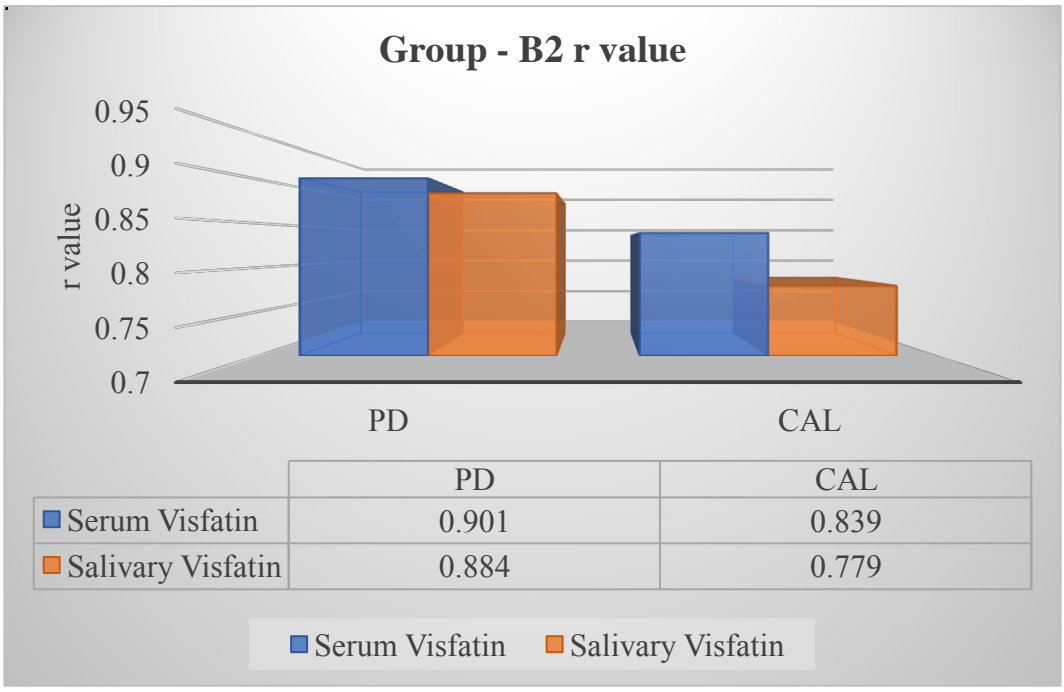
Graph – 10– Group – A - Correlation of Serum & Salivary Visfatin Levels with PD & CAL



Graph – 11– Group – B1 - Correlation of Serum & Salivary Visfatin Levels with PD & CAL



Graph – 12– Group – B2 - Correlation of Serum & Salivary Visfatin Levels with PD & CAL



DISCUSSION

More than two thirds of the world population suffer from any one of the chronic forms of periodontal disease according to epidemiological studies. Periodontal destruction is host-mediated by locally produced pro-inflammatory cytokines in response to the bacterial flora and its products. Adipokines are molecules mainly produced and released from adipocytes through exocytosis with multitude of functions among them with inflammatory association being the important.

Studies have been done previously to demonstrate the interplay between adipocytokines including leptin[58], resistin[59], visfatin[27] etc and periodontal pathology. In this study the aim was to ascertain the relationship between visfatin levels in serum and saliva in periodontal health and disease and the effect of non-surgical periodontal treatment on serum and salivary levels of visfatin.

Jia et al (2004)[60] suggested that Visfatin has potent pro-inflammatory and destructive properties and could play a role in persistence of inflammation through inhibition of apoptosis of neutrophils. Because visfatin exerts pro-inflammatory functions in multiple organ systems, this molecule could also correlate with chronic inflammation of periodontal tissue.

Sommer at al (2008)[61] reviewed the structure, regulation and function of visfatin and found that the relationship between visfatin with age and gender is controversial when taking into account multiple studies. So in order to eliminate these confounding factors we matched both groups in age (25-65 years old) and gender.

Visfatin has been traditionally linked with obesity and adiposity**. Haider et al (2006)**[62] analysed plasma visfatin in non-diabetic obese children and healthy controls. Plasma visfatin concentrations were almost twice elevated in obese compared to healthy controls. **Taskesen et al (2012)**[63] study on serum visfatin levels and adiposity in obese adolescents also revealed that obesity was associated with

elevated serum visfatin. A cross-sectional study[10] on Asian Indians also found that elevated visfatin levels were associated with obesity. Since Obesity may confound the results, we excluded obese patients and included only patients with normal BMI

Visfatin is also suggested to have been elevated in multiple systemic diseases like Type-2 DM[64], coronary artery disease[65], rheumatoid arthritis[66] etc. **Chang et al., (2011)**[67]reviewed and performed a meta-analysis of studies to identify the significance of visfatin in relation to overweight/obesity, type-2 diabetes mellitus, insulin resistance, metabolic syndrome and cardiovascular diseases. They found that Plasma visfatin concentrations were elevated in enrolled pateintswho were diagnosed with overweight/obesity, type 2 diabetes mellitus, metabolic syndrome, cardiovascular diseases and insulin resistance had a positive association with circulating visfatin levels. We excluded patients diagnosed with systemic diseases including metabolic, inflammatory and immunological origin to rule out the effect of these diseases influencing the results in our study.

Bai et al (2016)[68] found that serum visfatin levels were elevated in smokers when compared to controls. They found that in 3T3-L1 pre - adipocytes when ATP-dependent potassium (KATP) channel was blocked visfatin secretion was reduced but nicotine treatment increased visfatin secretion through activation of KATP channel. Studies[69] show that visfatin is highly released by adipose tissue, myometrium, fetal membranes, amnion, placenta, and adiposetissue.The decidual and amniotic membranes contained higher levels of PBEF mRNA at term. This lead us to exclude smokers and pregnant women from our study.

Tabari et al (2018)[70] studied visfatin expression in chronic periodontitis and aggressive periodontitis patients and found that a statistically significant positive correlation was found between inflammation grading and visfatin expression in

gingival tissues of aggressive periodontitis patients leading us to exclude patients with aggressive periodontitis in our study. In our study, there was no significant difference in terms of Age, Gender, BMI, Number of teeth and frequency of brushing in between the groups.

In our study we studied serum and salivary samples for measurement of visfatin levels. Previous studies[11,50,52] have utilized Gingival crevicular fluid (GCF) samples for measurement of visfatin levels. This was because GCF being in close contact with periodontal tissues would more accurately reflect the health and disease states. But the procedure to collect GCF samples is cumbersome.[71]

In our study we utilized salivary samples instead of GCF samples due to the following reasons. Saliva secretion is copious and its quantity is more when compared to GCF. The procedure for collection of saliva is simple and is non-invasive in nature making patient cooperation easy.[72] It can be done without specialist training. **Kaufmann et al (2000)**[73] have shown that saliva bears enzymes, biomarkers in detectable quantities similar to GCF and also that adipokines are present in measurable quantities in saliva including Visfatin.

Chiappin et al (2007)[74] have shown that polypeptide / plasma protein hormones due to its large molecular size cannot passively diffuse into saliva. In order for them to be present in saliva it may be from plasma leakage from oral diseased lesions, but also maybe from active transport from salivary glands or primary secretion. **Toda et al (2007)**[75] in their study reported a relationship between salivary adiponectin and an increased risk of cardiovascular diseases and non- insulin dependent diabetes mellitus. Micro et al studied salivary leptin as a possible diagnostic marker for salivary gland tumors. Studies[76,77] have shown that unstimulated whole saliva contains higher levels of protein concentration in

comparison to stimulated saliva. Due to this reason in our study we utilized unstimulated saliva samples.

In our study salivary visfatin levels have been elevated in chronic periodontitis patients when compared to controls. Salivary visfatin levels were 57.86±3.35 ng/ml in chronic periodontitis patients which was significantly higher when compared to the healthy controls (19.943±2.135 ng/ml). This high level may be from visfatin leakage due to edema or cell membrane damage in periodontitis, active secretion or transport from salivary glands.

Our results are similar to results obtained in study by **Tabari et al (2014)**[45] where visfatin levels were elevated in patients with chronic periodontitis when compared to healthy controls. **Ozcan et al (2015)**[78] also found that salivary visfatin levels were higher in patients with gingivitis and periodontitis than in healthy controls.

In our study Serum visfatin levels (38.1±2.89 ng/ml) were also higher in periodontitis group than in healthy controls (16.42±2.11 ng/ml). This is similar to the results obtained by **Turer at al (2016)**[80] where serum visfatin levels were higher in chronic periodontitis patients when compared to gingivitis patients or healthy controls. This is also similar to the results by **Pradeep et al (2011)**[11] who found that visfatin concentration in both GCF and serum were highest in patients with periodontitis followed by patients with gingivitis with the concentration in healthy controls being the least.

Nokhbehsaim et al (2013)[81] sought to study whether visfatin (NAMPT) may be the patho-mechanistic link between obesity and periodontal diseases. They examined the regulation of periodontal ligament cells (PDL) cells by visfatin and its production and relationship with other inflammatory mediators under infectious and

inflammatory condition via microarray and real time PCR analysis. Visfatin caused a significant upregulation of EGR1, MMP-1, SYT7, ITPKA, CCL2, NTM, IGF2BP3, and NRP1. It also increased MMP-1 and CCL-2 protein synthesis. MMP-1, a proteolytic molecule plays a critical role in modeling and remodeling of the extracellular matrix by degradation of COL1 and other types of collagen. Gingival levels of MMP-1 are enhanced at sites of periodontitis and can be reduced by periodontal treatment. CCL-2, a proinflammatory molecule, regulates the migration and infiltration of monocytes, memory T lymphocytes and natural killer cells and also seems to influence T-cell immunity. CCL2 levels were found to be elevated in GCF and gingiva from inflamed sites.

A study done by **Curat et al (2006)**[20] also showedthat visfatin production in PDL cells could be induced by periodontal pathogens, P. gingivalis and F. nucleatum, and some pro-inflammatory cytokines, IL-1β. This suggested that infectious and inflammatory signals can utilize visfatin as an intermediary for its destructive effects on periodontium. Studies[81] also suggest that visfatin can interfere with the regenerative capacity of periodontal ligament cells through the production of pro-inflammatory cytokines and matrix destructing cytokines.

Adya et al (2008)[82] demonstrated that visfatin causes an increase in expression and activity of MMP (matrix metalloproteinases) and it also causes down regulation of MMP inhibitors. Studies report that imbalance between MMP and their inhibitors plays a key role in the pathogenesis of periodontitis.[83] The above mentioned studies when considered, resulted in a hypothesis that there could be a relationship between visfatin and periodontitis.

In our study all of the clinical parameters including periodontal index (PI) [2.9933±0.5938 to 1.63±0.3958], gingival index (GI) [2.0633±0.1791 to

1.1113±0.2029], probing depth (PD) [5.270±0.8703 to 2.393±0.6736 mm] and clinical attachment loss (CAL) [6.1±0.8871 to 3.0933±0.6937 mm] reduced in response to non-surgical periodontal therapy. This is similar to the study done by **Raghavendra et al (2012)**[50] where all clinical parameters decreased in response to phase 1 periodontal therapy. However in a study done by **Tabari et al (2015)**[46] the reduction in CAL was not significant when compared to our study.

Ragavendra et al (2012)[50] in their study to evaluate the response of serum and GCF visfatin to nonsurgical periodontal therapy, found that serum and GCF visfatin reduced significantly 2 months after NSPT. The results of our study also show that the reduction in serum visfatin levels [38.1±2.8971 to 20.323±4.8444 ng/ml]2 months post periodontal therapy was statistically significant .

In our study salivary visfatin levels also reduced significantly post treatment [57.86±3.355 to 19.626±2.6122 ng/ml] in chronic periodontitis patients. Our results were in accordance with the study done by **Tabari et al (2015)**[51] which showed salivary visfatin levels decreased significantly after non-surgical periodontal treatment. The decrease in serum and salivary levels of visfatin post periodontal therapy could be possibly because of periodontal therapy leading to decreased infection resulting in reduction of inflammation and its mediating cytokines like IL-1β and IL-6 which in conjunction with visfatin play a role in chronic periodontitis.

Liu et al (2010)[84] showed that non-surgical periodontal therapy of chronic periodontitis patients is effective and results in bacterial load and pathogenic species levels reduction in periodontal pockets, elimination of inflammation and significant reduction of periodontal pockets depth. This also results in a shift of the local destructive response including expression levels of some pro-inflammatory factors IL-1β and IL-6.This was proven in a study done by **Mlachkova et al (2016)**[85] where

nonsurgical periodontal therapy in chronic periodontitis resulted in reduction of main inflammatory factors in gingival tissue - IL-1β and IL- 6.Another study by **Oh et al (2015)**[86] reinforced that GCF IL-1β levels were lowered after initial periodontal therapy.

Jia et al (2004)[60] identified that visfatin was upregulated in neutrophils by IL-1 and plays a role in the delayed neutrophil apoptosis of clinical and experimental sepsis through a caspase -3 and caspase -8 mediated mechanism. In another study **Nowell et al (2006)**[87]tried to assess whether PBEF/VF expression was directed by IL-6 trans- signaling both in-vivo and in- vitro. They used complementary DNA from RA (Rheumatoid arthritis) fibroblasts. They were able to demonstrate that PBEF / Visfatin is actively expressed during arthritis and it is regulated via IL-6 trans signaling and IL-6 related cytokine. This regulation of visfatin by the inflammatory cytokines could possibly be the reason of reduction of visfatin levels post nonsurgical periodontal therapy.

The reduction in serum and salivary levels of visfatin also correlated positively with reduction in clinical parameters like probing depth and clinical attachment loss. In the study done by **Abolfazliet al (2014)**[88]the though the clinical parameters like PI, GI, PD and CAL showed a correlation with serum and salivary levels of visfatin, the correlation relationship was not statistically significant. In the study done by **Tabari et al (2015)**[51]also there was no significant correlation between clinical parameters and salivary visfatin concentration. But in the study done by **Pradeep at al (2011)**[11], the serum Visfatin concentration had a statistically significant positive correlation with PD and CAL, with the relationship between CAL the strongest. The decrease in salivary visfatin levels post treatment to the levels of healthy controls could be because of the change in the microbial environment and the

subsequent reduction in inflammatory processes in the periodontal pockets. An additional reason for reduction in salivary visfatin levels could be because of the reduction in the source mainly cells responsible for visfatin production mainly leucocytes and macrophages.

Though serum visfatin post treatment levels (20.323 ± 4.8444 ng/ml) decreased when compared to pretreatment values, it was still higher when compared to normal controls (16.423±2.1148 ng/ml).However NSPT decreased salivary visfatin levels of chronic periodontitis patients post treatment (19.626±2.6122 ng/ml) comparable to the control group (19.943±2.1355). This decrease in post NSPT salivary visfatin levels to near control group is echoed by the study done by **Tabari et al (2015)[51].**

Cekmez et al (2011)[89] in their study on inflammatory cytokines and adipokines in neonatal sepsis found that visfatin could be used as a diagnostic marker similar to CRP, procalcitonin and IL-6 in neonatal sepsis. Visfatin has also been proposed biomarker with a potential prognostic value in cardiovascular diseases such as endothelial dysfunction, atherosclerosis and vascular damage.[90]

In our study visfatin is present in both serum and saliva measurable quantities in normal controls and chronic periodontitis patients. Its level in both serum and saliva is increased in chronic periodontitis patients. Our study also demonstrated that non-surgical periodontal therapy results in a statistically significant decrease of serum and salivary levels of visfatin in chronic periodontitis patients which also correlates with clinical parameters like PD and CAL. This shows that visfatin has the potential to be a target marker for chronic periodontitis to evaluate the response of treatment to periodontal diseases.

SUMMARY &CONCLUSION

The following major conclusions can be drawn from this study:

1. Serum and salivary visfatin levels were higher in patients with chronic periodontitis compared to periodontally healthy individuals.
2. Non-surgical periodontal therapy resulted in reduction of salivary and serum visfatin levels towards health.

The increasing levels of serum and salivary visfatin in chronic periodontitis and its subsequent reduction by non-surgical periodontal therapy holds visfatin in good stead as a marker to detect disease activity and a potential therapeutic target for assessment of effectiveness of treatment. Visfatin by its close association with systemic diseases could probably be the missing link associating periodontal diseases and systemic diseases. Evaluating the salivary levels of visfatin is non-invasive and easy and this could make it possible for it to become an acceptable alternative for the assessment and evaluating the systemic diseases which are associated with it.

Future long-term follow-up studies which involve large sample size is essential to validate the results of our study.

REFERENCES

1. Kirkwood KL, Cirelli JA, Rogers JE, Giannobile WV. Novel host response therapeutic approaches to treat periodontal diseases. Periodontol 2000. 2007;43:294-315
2. Van Dyke TE, Serhan CN. Resolution of inflammation: a new paradigm for the pathogenesis of periodontal diseases. J Dent Res. 2003 Feb;82(2):82-90
3. Preshaw PM, Taylor JJ. How has research into cytokine interactions and their role in driving immune responses impacted our understanding of periodontitis? J Clin Periodontol. 2011 Mar;38 Suppl 11:60-84
4. Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol. 2005 May;115(5):911-9; quiz 920
5. Samal B, Sun Y, Stearns G, Xie C, Suggs S, McNiece I. Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. Mol Cell Biol. 1994 Feb;14(2):1431-7
6. Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, Matsuki Y, Murakami M, Ichisaka T, Murakami H, Watanabe E, Takagi T, Akiyoshi M, Ohtsubo T, Kihara S, Yamashita S, Makishima M, Funahashi T, Yamanaka S, Hiramatsu R,Matsuzawa Y, Shimomura I. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science. 2005 Jan 21;307(5708):426-30
7. Sethi JK, Vidal-Puig A. Visfatin: the missing link between intra-abdominal obesity and diabetes? Trends Mol Med. 2005 Aug;11(8):344-7
8. Moschen AR, Kaser A, Enrich B, Mosheimer B, Theurl M, Niederegger H, Tilg H. Visfatin, an adipocytokine with proinflammatory and immunomodulating properties. J Immunol. 2007 Feb 1;178(3):1748-58

9. Ognjanovic S, Bao S, Yamamoto SY, Garibay-Tupas J, Samal B, Bryant-Greenwood GD. Genomic organization of the gene coding for human pre-B-cell colony enhancing factor and expression in human fetal membranes. J Mol Endocrinol. 2001 Apr;26(2):107-17
10. Sandeep S, Velmurugan K, Deepa R, Mohan V. Serum visfatin in relation to visceral fat, obesity, and type 2 diabetes mellitus in Asian Indians. Metabolism. 2007 Apr;56(4):565-70
11. Pradeep AR, Raghavendra NM, Prasad MV, Kathariya R, Patel SP, Sharma A. Gingival crevicular fluid and serum visfatin concentration: their relationship in periodontal health and disease. J Periodontol. 2011 Sep;82(9):1314-9
12. Mamali I, Roupas ND, Armeni AK, Theodoropoulou A, Markou KB, Georgopoulos NA. Measurement of salivary resistin, visfatin and adiponectin levels. Peptides. 2012 Jan;33(1):120-4
13. Kern PA, Di Gregorio GB, Lu T, Rassouli N, Ranganathan G. Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor-α expression. Diabetes. 2003 Jul 1;52(7):1779-85.
14. Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A. Resistin, an adipokine with potent proinflammatory properties. The Journal of Immunology. 2005 May 1;174(9):5789-95.
15. Bulcão C, Ferreira SR, Giuffrida FM, Ribeiro-Filho FF. The new adipose tissue and adipocytokines. Curr Diabetes Rev. 2006 Feb;2(1):19-28.
16. Kiguchi N, Maeda T, Kobayashi Y, Fukazawa Y, Kishioka S. Leptin enhances CC-chemokine ligand expression in cultured murine macrophage. Biochemical and biophysical research communications. 2009 Jul 3;384(3):311-5.

17. Tunkel J, Heinecke A, Flemmig TF. A systematic review of efficacy of machine-driven and manual subgingival debridement in the treatment of chronic periodontitis. Journal of Clinical Periodontology. 2002 Dec;29:72-81.
18. Ryan ME. Nonsurgical approaches for the treatment of periodontal diseases.Dent Clin North Am. 2005 Jul;49(3):611-36
19. Smiley CJ, Tracy SL, Abt E, Michalowicz BS, John MT, Gunsolley J, Cobb CM, Rossmann J, Harrel SK, Forrest JL, Hujoel PP. Systematic review and meta-analysis on the nonsurgical treatment of chronic periodontitis by means of scaling and root planing with or without adjuncts. The Journal of the American Dental Association. 2015 Jul 1;146(7):508-24.
20. Curat CA, Wegner V, Sengenès C, Miranville A, Tonus C, Busse R, Bouloumié A.Macrophages in human visceral adipose tissue: increased accumulation in obesity and a source of resistin and visfatin. Diabetologia. 2006 Apr;49(4):744-7
21. Garten A, Petzold S, Barnikol-Oettler A, Körner A, Thasler WE, Kratzsch J, Kiess W, Gebhardt R. Nicotinamide phosphoribosyltransferase (NAMPT/PBEF/visfatin)is constitutively released from human hepatocytes. Biochem Biophys Res Commun. 2010 Jan 1;391(1):376-81. doi: 10.1016/j.bbrc.2009.11.066
22. Luk T, Malam Z, Marshall JC. Pre-B cell colony-enhancing factor (PBEF)/visfatin: a novel mediator of innate immunity. J Leukoc Biol. 2008 Apr;83(4):804-16
23. Xiao K, Zou WH, Yang Z, Rehman ZU, Ansari AR, Yuan HR, Zhou Y, Cui L, Peng KM, Song H. The role of visfatin on the regulation of inflammation

and apoptosis in the spleen of LPS-treated rats. Cell Tissue Res. 2015 Feb;359(2):605-618.

24. Busso N, Karababa M, Nobile M, Rolaz A, Van Gool F, Galli M, Leo O, So A, De Smedt T. Pharmacological inhibition of nicotinamidephosphoribosyltransferase/visfatin enzymatic activity identifies a new inflammatory pathway linked to NAD. PLoS One. 2008 May 21;3(5):e2267.
25. Kang YS, Kang YG, Park HJ, Wee HJ, Jang HO, Bae MK, Bae SK. Melatonin inhibits visfatin-induced inducible nitric oxide synthase expression and nitric oxide production in macrophages. J Pineal Res. 2013 Oct;55(3):294-303
26. Li L, Yang G, Li Q, Tang Y, Yang M, Yang H, Li K. Changes and relations of circulating visfatin, apelin, and resistin levels in normal, impaired glucose tolerance, and type 2 diabetic subjects. Exp Clin Endocrinol Diabetes. 2006 Nov;114(10):544-8
27. Chen MP, Chung FM, Chang DM, Tsai JC, Huang HF, Shin SJ, Lee YJ. Elevated plasma level of visfatin/pre-B cell colony-enhancing factor in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab. 2006 Jan;91(1):295-9
28. Otero M, Lago R, Gomez R, Lago F, Dieguez C, Gómez-Reino JJ, Gualillo O. Changes in plasma levels of fat-derived hormones adiponectin, leptin, resistin and visfatin in patients with rheumatoid arthritis. Ann Rheum Dis. 2006 Sep;65(9):1198-201
29. Dahl TB, Yndestad A, Skjelland M, Øie E, Dahl A, Michelsen A, Damås JK, Tunheim SH, Ueland T, Smith C, Bendz B, Tonstad S, Gullestad L, Frøland SS, Krohg-Sørensen K, Russell D, Aukrust P, Halvorsen B. Increased

expression of visfatin in macrophages of human unstable carotid and coronary atherosclerosis: possible role in inflammation and plaque destabilization. Circulation. 2007 Feb 27;115(8):972-80

30. Ye SQ, Simon BA, Maloney JP, Zambelli-Weiner A, Gao L, Grant A, Easley RB, McVerry BJ, Tuder RM, Standiford T, Brower RG, Barnes KC, Garcia JG. Pre-B-cell colony-enhancing factor as a potential novel biomarker in acute lung injury. Am J Respir Crit Care Med. 2005 Feb 15;171(4):361-70
31. Gosset M, Berenbaum F, Salvat C, Sautet A, Pigenet A, Tahiri K, Jacques C. Crucial role of visfatin/pre-B cell colony-enhancing factor in matrix degradation and prostaglandin E2 synthesis in chondrocytes: possible influence on osteoarthritis. Arthritis Rheum. 2008 May;58(5):1399-409
32. Liu SW, Qiao SB, Yuan JS, Liu DQ. Association of plasma visfatin levels with inflammation, atherosclerosis and acute coronary syndromes (ACS) in humans. Clin Endocrinol (Oxf). 2009 Aug;71(2):202-7
33. Romacho T, Azcutia V, Vázquez-Bella M, Matesanz N, Cercas E, Nevado J, Carraro R, Rodríguez-Mañas L, Sánchez-Ferrer CF, Peiró C. Extracellular PBEF/NAMPT/visfatin activates pro-inflammatory signalling in human vascular smooth muscle cells through nicotinamide phosphoribosyltransferase activity. Diabetologia. 2009 Nov;52(11):2455-2463
34. Bae YH, Bae MK, Kim SR, Lee JH, Wee HJ, Bae SK. Upregulation of fibroblast growth factor-2 by visfatin that promotes endothelial angiogenesis. Biochem Biophys Res Commun. 2009 Feb 6;379(2):206-11
35. Carrero JJ, Witasp A, Stenvinkel P, Qureshi AR, Heimbürger O, Bárány P, Suliman ME, Anderstam B, Lindholm B, Nordfors L, Schalling M. Visfatin is increased in chronic kidney disease patients with poor appetite and correlates

negatively with fasting serum amino acids and triglyceride levels. Nephrology Dialysis Transplantation. 2009 Nov 30;25(3):901-6.

36. Park BS, Jin SH, Park JJ, Park JW, Namgoong IS, Kim YI, Lee BJ, Kim JG. Visfatin induces sickness responses in the brain. PLoS One. 2011 Jan 20;6(1):e15981.
37. Yang S, Ryu JH, Oh H, Jeon J, Kwak JS, Kim JH, Kim HA, Chun CH, Chun JS. NAMPT (visfatin), a direct target of hypoxia-inducible factor-2α, is an essential catabolic regulator of osteoarthritis. Ann Rheum Dis. 2015 Mar;74(3):595-602
38. Naz Sh, Sandhu QSh, Akhtar A, Zafar U, Khalid A, Saeed M. Serum Levels of Visfatin and Interleukin-6 in Non-Obese Versus Obese Men with Coronary Artery Disease. J Coll Physicians Surg Pak. 2017 Feb;27(2):71-74
39. Srinivasan M, Meadows ML, Maxwell L. Assessment of Salivary Adipokines Resistin, Visfatin, and Ghrelin as Type 2 Diabetes Mellitus Biomarkers. Biochem Res Int. 2018 Feb 1;2018:7463796
40. Yapıcı Yavuz G, Şimşek Kaya G, Kızıltunç A. Analysis of synovial fluid visfatin level in temporomandibular joint disorders. Cranio. 2018 Feb 1:1-8
41. Nogueira AV, Nokhbehsaim M, Eick S, Bourauel C, Jäger A, Jepsen S, Cirelli JA, Deschner J. Regulation of visfatin by microbial and biomechanical signals in PDL cells. Clin Oral Investig. 2014 Jan;18(1):171-8
42. Özcan E, Saygun NI, Serdar MA, Kubar A, Bengi VU. Porphyromonas gingivalis and Epstein-Barr Virus Are Associated With Increased Levels of Visfatin in Gingival Crevicular Fluid. J Periodontol. 2016 Apr;87(4):443-51
43. Özcan E, Saygun NI, Ilıkçı R, Karslıoğlu Y, Muşabak U, Yeşillik S. Increased visfatin expression is associated with nuclear factor-kappa B and

phosphatidylinositol 3-kinase in periodontal inflammation. Clin Oral Investig. 2017 May;21(4):1113-1121

44. Pradeep AR, Raghavendra NM, Sharma A, Patel SP, Raju A, Kathariya R, Rao NS, Naik SB. Association of serum and crevicular visfatin levels in periodontal health and disease with type 2 diabetes mellitus. J Periodontol. 2012 May;83(5):629-34
45. Tabari ZA, Azadmehr A, Nohekhan A, Naddafpour N, Ghaedi FB. Salivary visfatin concentrations in patients with chronic periodontitis. J Periodontol. 2014 Aug;85(8):1081-5
46. Özcan E, Saygun NI, Serdar MA, Kurt N. Evaluation of the salivary levels of visfatin, chemerin, and progranulin in periodontal inflammation. Clin Oral Investig. 2015 May;19(4):921-8
47. 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 Jul;86(7):882-9
48. Sarhat E, Mohammed I, & Hamad A. (2017). Biochemistry of Serum and Saliva in Obese Individuals with Periodontitis : Case - Control Study. JODR. 4. 2-11
49. Yu, T.H., Wang, C.P., Hung, W.C., Cheng, Y.A., Chen, C.Y. and Lin, L.W., Plasma Levels of Visfatin are Associated with Periodontal Disease Severity in Coronary Artery Disease Patients. E-Da Medical Journal 2017;4(1):10-17
50. Raghavendra NM, Pradeep AR, Kathariya R, Sharma A, Rao NS, Naik SB. Effect of non-surgical periodontal therapy on gingival crevicular fluid and

serum visfatin concentration in periodontal health and disease. Dis Markers. 2012;32(6):383-8

51. Tabari ZA, Ghaedi FB, Azadmehr A, Nohekhan A, Tabrizi MA, Ardakani MR, Naddafpour N. Salivary Visfatin Concentration in Response to Non-surgical Periodontal Therapy. J Clin Diagn Res. 2015 Apr;9(4):ZC05-8
52. Türer ÇC, Balli U, Güven B, Çetinkaya BÖ, Keleş GÇ. Visfatin levels in gingival crevicular fluid and serum before and after non-surgical treatment for periodontal diseases. J Oral Sci. 2016;58(4):491-499
53. 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. 2002.
54. 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.
55. Tursky S, Gilmore ND, Glickman I.Reduced plaque formation by the chlormethyl analogue of vit. c. J Periodontol. 1970; 41(1):41-3
56. LoeH, silness J.Periodontal disease in Pregnancy. Acta Odontol scand 1963; 21: 533- 51.
57. Ainamo, J & Bay I. Problems & proposals for recording gingivitis & plaque. Int Dent J 25; 229-235, 1975.
58. Jain H, Mulay S. Relationship between periodontitis and systemic diseases: Leptin, a new biomarker?. Indian Journal of Dental Research. 2014 Sep 1;25(5):657.

59. Devanoorkar A, Kathariya R, Guttiganur N, Gopalakrishnan D, Bagchi P. Resistin: a potential biomarker for periodontitis influenced diabetes mellitus and diabetes induced periodontitis. Disease markers. 2014;2014.

60. Jia SH, Li Y, Parodo J, Kapus A, Fan L, Rotstein OD, Marshall JC. Pre-B cell colony-enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis. J Clin Invest. 2004 May;113(9):1318-27

61. Sommer G, Garten A, Petzold S, Beck-Sickinger AG, Blüher M, Stumvoll M, Fasshauer M. Visfatin/PBEF/Nampt: structure, regulation and potential function of a novel adipokine. Clinical Science. 2008 Jul 1;115(1):13-23.

62. Haider DG, Holzer G, Schaller G, Weghuber D, Widhalm K, Wagner O, Kapiotis S, Wolzt M. The adipokine visfatin is markedly elevated in obese children. Journal of pediatric gastroenterology and nutrition. 2006 Oct 1;43(4):548-9.

63. Taşkesen D, Kirel B, Us T. Serum visfatin levels, adiposity and glucose metabolism in obese adolescents. Journal of clinical research in pediatric endocrinology. 2012 Jun;4(2):76.

64. Esteghamati A, Alamdari A, Zandieh A, Elahi S, Khalilzadeh O, Nakhjavani M, Meysamie A. Serum visfatin is associated with type 2 diabetes mellitus independent of insulin resistance and obesity. Diabetes Research and Clinical Practice. 2011 Feb 1;91(2):154-8.

65. Fadaei R, Parvaz E, Emamgholipour S, Moradi N, Vatannejad A, Najafi M, Doosti M. The mRNA expression and circulating levels of visfatin and their correlation with coronary artery disease severity and 25-hydroxyvitamin D. Hormone and Metabolic Research. 2016 Apr;48(04):269-74.

66. Polyakova JV, Simakova ES, Zavodovsky BV, Seewordova LE, Zborovskaya IA. AB0249 Serum Visfatin Determination in Rheumatoid Arthritis. Annals of the Rheumatic Diseases. 2014 Jun 1;73:886.
67. Chang YH, Chang DM, Lin KC, Shin SJ, Lee YJ. Visfatin in overweight/obesity, type 2 diabetes mellitus, insulin resistance, metabolic syndrome and cardiovascular diseases: a meta-analysis and systemic review. Diabetes/metabolism research and reviews. 2011 Sep;27(6):515-27.
68. Bai XJ, Fan LH, He Y, Ren J, Xu W, Liang Q, Li HB, Huo JH, Bai L, Tian HY, Fan FL. Nicotine may affect the secretion of adipokines leptin, resistin, and visfatin through activation of KATP channel. Nutrition. 2016 Jun 1;32(6):645-8.
69. Ognjanovic S, Bryant-Greenwood GD. Pre-B-cell colony-enhancing factor, a novel cytokine of human fetal membranes. American journal of obstetrics and gynecology. 2002 Oct 1;187(4):1051-8.
70. Tabari ZA, Keshani F, Sharbatdaran M, Banishahabadi A, Nejatifard M, Ghorbani H. Visfatin expression in gingival tissues of chronic periodontitis and aggressive periodontitis patients: An immunohistochemical analysis. Dental research journal. 2018 Mar;15(2):104.
71. Todorovic T, Dozic I, Vicente-Barrero M, Ljuskovic B, Pejovic J, Marja novic M, et al. Salivary enzymes and periodontal disease. Med Oral Patol Oral Cir Bucal 2006;11:115-9 .
72. Ozmeric N. Advances in periodontal disease markers. Clinica chimica acta. 2004 May 1;343(1-2):1-6.
73. Kaufman E, Lamster IB. Analysis of saliva for periodontal diagnosis: a review. Journal of clinical periodontology. 2000 Jul;27(7):453-65.

74. Chiappin S, Antonelli G, Gatti R, Elio F. Saliva specimen: a new laboratory tool for diagnostic and basic investigation. Clinica chimica acta. 2007 Aug 1;383(1-2):30-40.

75. Toda M, Tsukinoki R, Morimoto K. Measurement of sali- vary adiponectin levels. Acta Diabetologica 2007;44:20-2

76. Schapher M, Wendler O, Groschl M, Schafer R, Iro H, Zenk J. Salivary leptin as a candidate diagnostic marker in salivary gland tumors. Clin Chem 2009;55:914-22

77. 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.

78. Özcan E, Saygun NI, Serdar MA, Kurt N. Evaluation of the salivary levels of visfatin, chemerin, and progranulin in periodontal inflammation. Clinical oral investigations. 2015 May 1;19(4):921-8.

79. Amid R, Torshabi M, Yahyazadeh N, Yousefi N, Ekhlasmand Kermani M. Comparison of salivary Visfatin in patients with periodontitis and peri-implantitis in an Iranian population. Journal of Periodontology & Implant Dentistry. 2017 May 4;8(2):37-42.

80. Türer ÇC, Balli U, Güven B, Çetinkaya BÖ, Keleş GÇ. Visfatin levels in gingival crevicular fluid and serum before and after non-surgical treatment for periodontal diseases. Journal of oral science. 2016;58(4):491-9.

81. Nokhbehsaim M, Eick S, Nogueira AV, Hoffmann P, Herms S, Fröhlich H, Jepsen S, Jäger A, Cirelli JA, Deschner J. Stimulation of MMP-1 and CCL2 by NAMPT in PDL cells. Mediators of inflammation. 2013;2013.

82. Adya R, Tan BK, Punn A, Chen J, Randeva HS. Visfatin induces human endothelial VEGF and MMP-2/9 production via MAPK and PI3K/Akt signalling pathways: novel insights into visfatin-induced angiogenesis. Cardiovascular research. 2007 Dec 18;78(2):356-65.

83. Sapna G, Gokul S, Bagri-Manjrekar K. Matrix metalloproteinases and periodontal diseases. Oral diseases. 2014 Sep;20(6):538-50.

84. Liu YC, Lerner UH, Teng YT. Cytokine responses against periodontal infection: protective and destructive roles. Periodontology 2000. 2010 Feb;52(1):163-206.

85. Mlachkova A, Dosseva-Panova V, Popova C, Kicheva M. Effectivenessof non-surgical periodontal therapy by gingival expression of IL-1β AND IL-6. Journal of IMAB: Annual Proceeding (Scientific Papers). 2016 Jan 1;22(3):1257-60.

86. Oh H, Hirano J, Takai H, Ogata Y. Effects of initial periodontal therapy on interleukin-1β level in gingival crevicular fluid and clinical periodontal parameters. Journal of oral science. 2015;57(2):67-71.

87. Nowell MA, Richards PJ, Fielding CA, Ognjanovic S, Topley N, Williams AS,Bryant-Greenwood G, Jones SA. Regulation of pre-B cell colony-enhancing factor by STAT-3-dependent interleukin-6 trans-signaling: implications in the pathogenesis of rheumatoid arthritis. Arthritis Rheum. 2006 Jul;54(7):2084-95

88. Abolfazli N, Jabali S, Saber FS, Babaloo Z, Shirmohammadi A. Effect of non-surgical periodontal therapy on serum and salivary concentrations of visfatin in patients with chronic periodontitis. Journal of dental research, dental clinics, dental prospects. 2015;9(1):11.

89. Cekmez F, Canpolat FE, Çetinkaya M, Aydinöz S, Aydemir G, Karademir F, Ipcioglu OM, Sarici SÜ. Diagnostic value of resistin and visfatin, in comparison with C-reactive protein, procalcitonin and interleukin-6 in neonatal sepsis. European cytokine network. 2011 Jul 1;22(2):113-7.
90. Romacho T, Sánchez-Ferrer CF, Peiró C. Visfatin/Nampt: an adipokine with cardiovascular impact. Mediators of inflammation. 2013;2013.

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/40/2016

Date: 05.11.2016

TO WHOMSOEVER IT MAY CONCERN

Principal Investigator: Dr. D. Surya

Title: Acomparative study on salivary and serum levels of Visfatin in periodontally healthy and chronic periodontitis patients before and after non-surgicalperiodontal 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 25.10.16.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.
(Tk) Namakkal (Dt),
Tamil Nadu.

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/40/2016 Date: 05.11.2016

TO WHOMSOEVER IT MAY CONCERN

Principal Investigator: Dr. D. Surya

Title: Acomparative study on salivary and serum levels of Visfatin in periodontally healthy and chronic periodontitis patients before and after non-surgicalperiodontal 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 25.10.16.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.
(Tk) Namakkal (Dt),
Tamilnadu.