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A Comparative Study on Serum Adiponectin and Leptin levels in Periodontitis Patients with and without Diabetes Mellitus Type2

A Thesis

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Vean

Summary

Periodontal diseases are initiated by microbial plaque, which accumulates in the sulcular region and induces an inflammatory response. Recently, studies found that periodontitis might be related to several systemic diseases especially diabetes mellitus. In recent years, there has been intense interest in the role of the adipose tissue derived substances that named adipokines in the inflammatory diseases of the human being including the inflammatory periodontitis. This study was performed to evaluate the serum level of leptin and adiponectin in periodontitis patients with and without type 2 diabetes mellitus (T2DM), to determine the association between serum level of the biochemical markers (leptin and adiponectin) with clinical periodontal parameters, and to investigate the correlation between leptin and adiponectin.

Sixty subjects with periodontitis consist of (30 periodontitis patients and 30 periodontitis+T2DM patients) their ages range from 32-64 years and 25 apparently healthy volunteers their ages and sexes were matched with the patients were participated in this study. Periodontal parameters used in this study were plaque index, gingival index, probing pocket depth, clinical attachment level and bleeding on probing. Blood samples were collected from all patients and controls, and then serum was separated from blood to estimate the levels of leptin and adiponectin by enzyme-linked immunosorbent assay.

The present data revealed a significant elevation ($p<0.01$) in mean serum level of leptin in periodontitis group and periodontitis+T2DM group (25.89±5.52 ng/ml and 32.16±7.78 ng/ml, respectively) in comparison to that in healthy control (16.66±3.93 ng/ml) moreover, the comparison between two groups of patients showed that the mean level of leptin was increase in periodontitis+T2DM group but statistically not significant ($p>0.05$). On the other hand, there is a significant decrease ($p<0.001$) in mean serum level of adiponectin in both patients groups (60.08±9.61ng/ml and 50.10± 7.64 ng/ml, respectively) when compared to control group (77.57±10.80 ng/ml), additionally, there is slight significant reduction in the

mean serum level of adiponectin in periodontitis patients without T2DM when compared to those patients with T2DM, ($p<0.05$).

The ratio of leptin\adiponectin was significantly higher among patients groups (0.43±0.08 and 0.64±0.03) when compared with the ratio in the control group (0.21±0.02), ($P<0.01$). Interestingly negative significant correlation was noticed between leptin and adiponectin in periodontitis patients ($r=-0.325$, $p=0.011$) and also in periodontitis+T2DM group ($r=-0.434$, $p=0.017$).

Regarding the correlation between serum leptin and adiponectin levels and clinical periodontal parameters, these findings did not observe any significant correlation between serum level of leptin, adiponectin, and ratio of leptin/adiponectin with clinical periodontal parameters ($p>0.05$). In conclusion this study demonstrated that serum levels of leptin and adiponectin play a crucial role in pathogenesis of periodontitis with and without T2DM, and the relative leptin/adiponectin ratio appears to be indicative of disease occurrence. Moreover imbalance between pro and anti-inflammatory mediators could be involved in the initiation and progression of periodontitis and is indicative of a stronger systemic proinflammatory state in disease.

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List of Abbreviations

Abbreviations	Full name
ADA	American Diabetes Association
AMI	acute myocardial infarction
AMP	Adenosine monophosphate
BOP	Bleeding on probing
BMI	Body Mass Index
C.E.J	cemento-enamel junction
CAL	clinical attachment level
DM	Diabetes mellitus
ELISA	Enzyme linked immunosorbent assay
FBG	fasting blood glucose
GCF	gingival crevicular fluid
GI	Gingival index
HMW	High Molecular Weight
HRP	horseradish peroxidase
IFN-γ	Interferon- gamma
IL-1	Interleukin-1
IL-11	Interleukin-11
IL-10	Interleukin-10
IL-12	Interleukin-12
IL-1β	Interleukin 1- beta
IL-2	Interleukin-2
IL-6	Interleukin-6
INOS	inducible nitric oxide synthase
LMW	low molecular weight
LPS	Lipopolysaccharide
MAMPs	microbial-associated molecular patterns
Ml	Milliliter
MMPs	Matrix metalloproteinases
MMW	medium molecular weight
mRNA	Messenger ribonucleic acid
NEFA	non- esterified fatty acid
NF-kB	Nuclear factor kappa B
Ng	Nanograms
NHANES	National Health and Nutrition Examination Survey
NIDDM	Non-insulin dependent diabetes mellitus
NK	natural killer cells
NLR	intracellular NO-like receptors
Nm	Nano meter

ObR	Leptin receptors
OD	Optical density
PK	Protein kinase
PI	Plaque Index
PMNs	Poly morphonuclear neutrophils
PPD	Probing Pocket Depth measurement
PRRs	pattern recognition receptors
Rpm	Revolutions Per Minute
T1DM	Diabetes MellitusType1
T2DM	Diabetes Mellitus Type 2
TLRs	Toll-like receptors
TMB	Tetramethyl-benzidine
TNF-α	Tumor necrosis factor alpha
VLCD	very-low-calorie diet
WHO	World Health Organization
Ml	Microliter
KDa	Kilo Dalton

Chapter One

Introduction and Literatures Review

Introduction

1.1 Introduction

Periodontal diseases are comprised of a group of inflammatory conditions that result in the destruction of the supporting structures of the dentition, leading to loss of the connective tissue attachment and alveolar bone, resulting in loss of the teeth. Though the microorganisms are implicated as the etiologic agent to bring about inflammatory lesion, the chemical mediators of inflammation play a pivotal role in the loss of connective tissue, as well as supporting alveolar bone (Carenza, 2009). Cytokines like Interleukin 1- beta (IL- 1ß), Tumor necrosis factor alpha (TNF- α) and adipocytokines like adiponectin and leptin has been shown to orchestrate the host response to infection and inflammatory stimuli (Gesta *et al.*, 2007). However; evidences indicate that periodontitis may have profound effects on systemic health. Epidemiologic studies reported that greater prevalence or severity of periodontitis was seen in diabetic individuals than in non-diabetic subjects (Taylor and Borgnakke, 2008).

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycaemia due to the defective secretion or activity of insulin. DM present with the classical triad of symptoms such as polydypsia, polyuria and polyphagia. This is often accompanied by chronic fatigue and loss of weight. Complications of DM include retinopathy, nephropathy, neuropathy, and cardiovascular disease (Khader *et al*., 2008). Periodontitis is referred to as the fifth most common complication of diabetes. Clinical studies have demonstrated a higher prevalence of periodontitis in diabetic patients. However, periodontal disease and diabetes have a number of common pathways in their pathogenesis; both diseases are polygenic disorders with some degree of immunoregulatory dysfunction (Soskolne and Klinger., 2001). With the start of the current century, an interested was increased about the role of the adipose tissue that produces and releases a variety of inflammatory factors, including

adiponectin, resistin, leptin and visfatin, as well as cytokines such as TNF-α and IL-6. These factors and cytokines are thought to play a role in inflammation and immune responses (Lago *et al*., 2007).

Adipokines are bioactive mediators released from the adipose tissue including adipocytes and other cells present within fat tissues. These include several novel and highly active molecules released abundantly by adipocytes like leptin, resistin, adiponectin and visfatin (Tilg and Moschen., 2006).

Adiponectin , a 30-kDa protein, mainly secreted by adipocytes, has anti-inflammatory, antidiabetic, and anti-atherogenic properties, which circulates in high concentrations in the blood. Adiponectin levels are decreased in individuals with obesity, DM type2 and cardiovascular disease. Adiponectin inhibits osteoclast formation stimulated by lipopolysaccharide (LPS) from *Actinobacillus actinomycetemcomitans*. Regulation of adiponectin is provided by inflammatory cytokines such as IL-6 and TNF-α (Yamaguchi *et al*., 2007).

Leptin is a 16-kDa nonglycosylated peptide hormone. It is synthesized mainly in adipocytes and in minor quantities by T cell, osteoblast and gastric epithelium. Leptin has been classified as a cytokine as it shows structural similarities to the IL-6 and IL-11 (Rosa *et al*., 2010).

The overall increase in leptin during infection and inflammation indicates that leptin is a part of the immune response and host defense mechanisms. Since, leptin has a role in the inflammatory response. An increase in leptin level in saliva of healthy gingiva may be a host defense mechanism as during sepsis (Sanchez and Romero., 2001).

Aims of Study

This study was carried out to shed light on the following:

1. The role of serum adiponectin and leptin in the pathogenesis of periodontitis with and without Diabetes Mellitus Type 2 .
2. The relationship between serum levels of the adiponectin and leptin with clinical periodontal parameters (plaque index, gingival index, bleeding on probing, pocket depth and clinical attachment level).
3. The correlation between adiponectin and leptin levels.

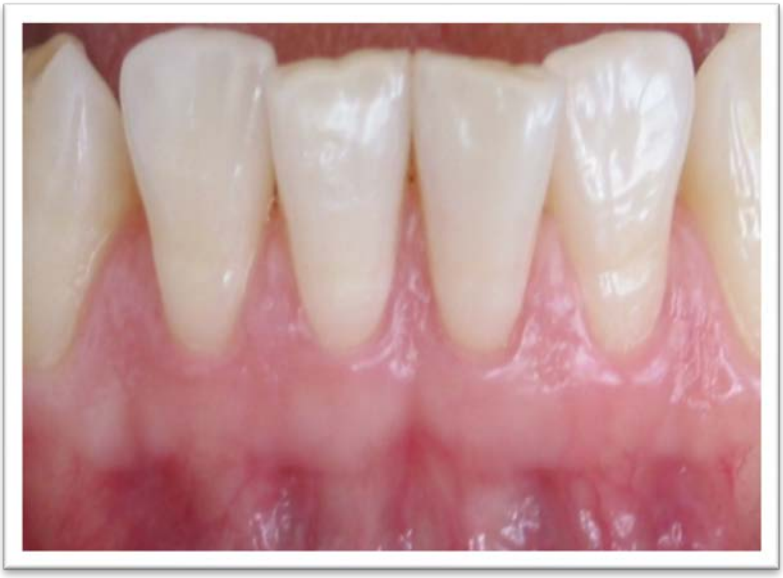
Literature Review

1.2 Periodontal diseases

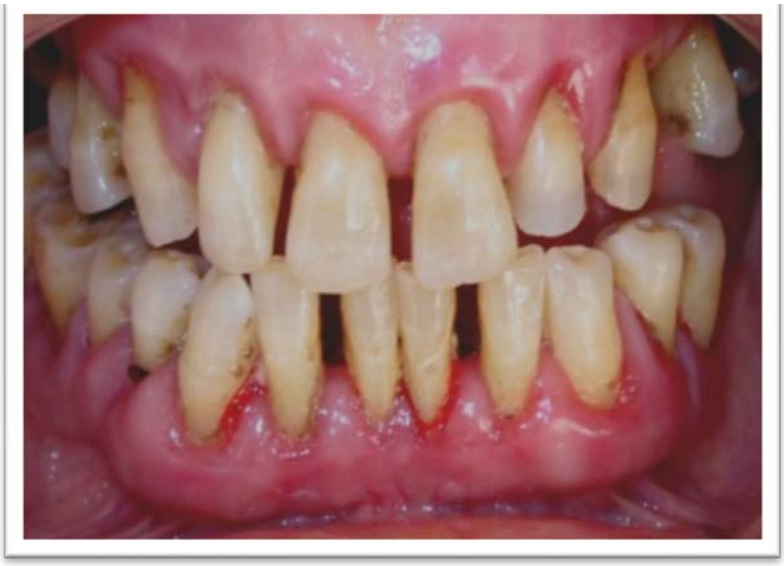
Oral diseases represent a common public health problem in a world like dental caries and periodontal diseases being the most prevalent. Periodontal diseases are a group of infectious diseases affecting the supporting tissue of the teeth, having common clinical manifestations of inflammation (Armitage, 2004).

These diseases are assumed to be associated with pathogenic bacteria species colonizing sub-gingival regions and result from the complex interaction between bacterial infection and host response, modified by environmental factors (Nishihara and Koseki, 2004).

Inflammation of the marginal gingival tissues is a common condition and its extent and severity can be variable. This condition, known as gingivitis can be modified by systemic and local influences and is plaque induced. It can be reversed if improved oral hygiene measures are introduced. Periodontitis is the result of a response of the host to bacterial aggregations on the tooth surfaces as shown in figure (1.1). The outcome of this is an irreversible destruction of the connective tissue attachment, which results in the periodontal pocket formation and eventual loss of alveolar bone (Moutsopoulos and Madianos, 2006).



A. Clinical picture of healthy teeth and periodontium . gingiva is characterised by a coral-pink colour, there is no sign of oedema or bleeding and the gingiva is tightly surrounding the tooth.



B. Clinical picture of a case of severe chronic periodontitis with T2DM. Note the pronounced gingival inflammation, bleeding, and swelling, but also gingival recession. There is visible plaque accumulation at the necks of the teeth and calculus deposits are also easily detectable.

Figure (1.1): picture showed different between (A) healthy teeth and (B) periodontitis (Ertugrul, 2013).

1.2.1 Periodontitis

The word "periodontitis" comes from peri (around) odont (tooth) and itis ("inflammation"). is a set of inflammatory diseases affecting the periodontium, i.e., the tissues that surround and support the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both (Caranza, 2009).

The clinical feature that distinguishes periodontitis from gingivitis is the presence of clinically detectable attachment loss, this often is accompanied by periodontal pocket formation and changes in the density and height of subjacent alveolar bone. In some cases, the recession of the marginal gingiva may accompany attachment loss (Van Dyke, 2007).

Chronic periodontitis is the most common of periodontitis can affect a few teeth or the whole dentition in the oral cavity. Chronic periodontitis is strongly associated with bacterial infection and severe inflammation (Zadik *et al*, 2008). Wiebe and Putnins mentioned that chronic periodontitis is a prevalent condition, occurring mostly in adults, and subgingival calculus is most commonly found. It is generally a slowly progressing disease, but can also have periods of rapid destruction. Chronic periodontitis can be divided into generalized and localized forms depending on the percentage of sites involved. The severity of chronic periodontitis can also be divided in to slight, moderate and severe chronic periodontitis based on the amount of CAL (Wiebe and Putnins, 2000).

1. 2.1.1 Epidemiology

Periodontitis is widely regarded as the second most common disease worldwide, after dental decay, and in the United States has a prevalence of 30–50% of the population, but only about 10% have severe forms. Like other conditions that are intimately related to access to hygiene and basic medical monitoring and care

(Pihlstrom *et al*., 2005). Periodontitis tends to be more common in economically disadvantaged populations or regions. Susin and associates reported that periodontitis is a major concern in Brazil with prevalence as high as 79% depending on the adopted diagnostic criteria (Susin *et al.*, 2004).

Generally, in Yemenite, North-African, South Asian, or Mediterranean origin have higher prevalence of periodontal disease than individuals from European descent individuals living in East Asia (e.g. Japan, South Korea and Taiwan) have the lowest incident of periodontal disease in the world. This could be attributed to genetic predisposition as well as cultural-behavioral differences (e.g, smoking, oral hygiene, and access to dental treatment) between populations (Zadik *et al*., 2008).

1.2.1.2 Risk factors

Periodontitis presents a multifactorial etiology which makes its study complex. There are some denominators such as stress, smoking, DM, older age, osteoporosis, genetic predisposition, low educational or socioeconomic conditions, and the presence of infections with certain bacteria and viruses. Genetic polymorphisms represent an important component in susceptibility to periodontitis (Rutger *et al.,* 2003). Moreover, accumulating evidence demonstrates that genetic variations in genes that codify for proinflammatory cytokines could affect the systemic inflammatory response in periodontitis patients (Kinane and Attstrom, 2005; Lim *et al.,* 2007).

1.2.1.3 Pathogenesis

The development of periodontitis is related with a specific group of predominantly Gram-negative, anaerobic or microaerophilic bacteria. They colonize on the root surface in the subgingival area (Page and Beck, 1997). The key periodontal pathogens in chronic periodontitis are (*Porphyromonas gingivalis, Tannerella forsythia* and *Treponema denticola*). whereas those in localized

aggressive periodontitis are *Aggregatibacter* (formerly termed *Actinobacillus*) *actinomycetemcomitan*s (Van Dyke and Serhan, 2003).

Although bacteria are essential, the bacteria alone are not sufficient for the disease to occur. It was well- recognized that host responses to the periodontal pathogens and their virulence factors play an important role in the pathogenesis of periodontitis (Gardy *et al*., 2009).

After colonization on the gingival sulcus by periodontal bacteria, the bacteria release their products, for example, lipopolysaccharide (LPS) which is well known virulence factor of Gram -negative bacteria. LPS has the potential to interact with nearly all cell types in periodontal tissues including immune cells and resident cells and thereby initiating a defense mechanism. The initial immune response in periodontal disease is characterized by the action of the innate immune system which, in this context, consists of the gingival epithelium, fibroblasts, neutrophils, dendritic cells, and monocytes/macrophages (Teng, 2006).

In fact, innate host recognition of LPS is a key initiating event for the subsequent clearance of Gram-negative bacteria from infected host tissues (Jain and Darveau, 2010). A group of receptors called pattern recognition receptors (PRRs) which include cell surface Toll-like receptors (TLRs) and intracellular NO-like receptors (NLRs) are responsible for the detection of microbial-associated molecular patterns (MAMPs), i.e. LPS, and thereby leading to cellular activation (Pathirana *et al*., 2010; Taylor, 2010). However; little knowledge is yet known how NLRs sense oral bacteria (Bostanci *et al.*, 2009).

In the gingival epithelium, the binding of LPS to TLR of nearby cells induces the production of cytokines and chemokines resulting in the expression of adhesion molecules, increased permeability of gingival capillaries and chemotaxis of polymorphonuclear neutrophils (PMNs) through the junctional epithelium and into the gingival sulcus to phagocyte bacteria. One important component of innate immunity that plays a vital role in periodontal disease is monocytes (Teng, 2006).

In response to inflammatory signals, monocytes can migrate quickly to sites of infection in the tissues and differentiate into macrophages which can effectively capture invading pathogens. The phagocytosis of bacteria by macrophages results in cytokine secretion and antigen-presentation to induce a more effective adaptive immunity (Liu *et al*., 2010).

Later on, if the plaque biofilm matures further, the pathogenic species developing in the periodontal pockets release an array of virulence factors, antigens or by products particularly LPS, into the pocket junctional epithelium, blood vessels and deeper connective tissues of periodontium (Slots and Ting, 1999).

This leads to a chronic inflammatory response characterized by dysregulation of immune-bacteria interactions where the infected tissues/cells are overwhelmed by the persistent pathogens accompanied with continuous and excessive production of potent proinflammatory cytokines (i.e. IL-1β, TNF-α, IFN-γ and IL-6) (Okada and Murakami, 1998; Preshaw, 2008).

Consequently, as the disease progresses to the more advanced stage(s), the specific cytokines and chemokines produced by innate immune response direct the host response towards a robust cell-mediated adaptive immunity. The dominant perivascular T-cell/macrophage infiltrate is observed in the connective tissues (Ohlrich *et al*., 2009).

If this T-cell response does not overcome the bacterial challenge, the disease proceeds to B-cell/plasma-cell dominated lesion. The production of antibodies by B-cell/plasma may be protective and control the infection (Gemmell *et al*., 2002; 2007).

The characteristics of chronic periodontitis is mediated by the B-cell/plasma cell response, the immunohistological features of chronic periodontitis are characterized by an apical migration of plaque on the root surface, accompanied by subgingival calculus formation. At this stage, a predominance of plasma cell infiltrates with few macrophages is observed in connective tissue (Kinane and Bartold, 2007). There is an alteration in appearance and reduction in the number of

local fibroblasts with the formation of the encapsulated fibrous band surrounding the body of the lesion. High levels of IL-1 and IL-6 produced from infiltrating cells lead to the production of matrix metalloproteinases (MMPs), especially by fibroblasts which, in turn, results in further attachment loss and bone resorption (Gemmell and Seymour, 1998; Nishikawa *et al*., 2002; Smith *et al*., 2010).

Clearly, periodontal disease is a multi-factorial disorder. The primary cause of periodontal disease is plaque bacteria but the disease progression is modified by an individual's susceptibility (Kinane *et al*., 2007; Preshaw, 2008). A wide variety of determinants and factors, either environmental or acquired, e.g. smoking, diabetes, systemic diseases, genetic factors, microbial composition of dental plaque are known to influence the host response (Nunn, 2003; Kinane and Bartold, 2007; Kinane *et al*., 2007).

Therefore, these factors could subsequently have certain effects on the disease initiation and progression (Grossi, 2000; Chavarry *et al*., 2009). So that, a complete understanding how diabetes contributes periodontal disease progression could be a key to establish rational diagnostic and therapeutic strategies for periodontal disease associated with diabetes (Anner *et al*., 2010).

1.2.1.4 Periodontitis and Systemic Diseases

In recent years, there has been intense interest in potential associations between periodontal disease and various chronic systemic diseases and conditions (Linden *et al*. 2012). Prospective cohort studies, which show that periodontal disease is associated with an increased risk of premature death from any cause, suggest the hypothesis that periodontitis may be a risk factor for other diseases (DeStefano *et al*. 1993, Garcia *et al*. 1998). A large body of research work has investigated periodontitis as an independent risk factor for atherosclerosis including stroke (Wu *et al*. 2000) and coronary heart disease (Buhlin *et al*. 2011); adverse pregnancy outcome and diabetes (Preshaw *et al*. 2012).

1.3 Diabetes Mellitus

Diabetes mellitus (DM), is a group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because the cells do not respond to the insulin that is produced (Shoback *et al*., 2011). Diabetes presents as a triad of symptoms including polydypsia, polyphagia and polyuria. These symptoms are the direct result of hyperglycemia and the resultant osmotic imbalance (Lawrence *et al*., 2008). The five major complications of diabetes are retinopathy, nephropathy, neuropathy, circulatory abnormalities and altered wound healing. The periodontium is also a target for diabetic damage, and Periodontal diseases has been suggested to be the sixth classic complication of diabetes (Loe, 1993; Grossi *et al.*, 1995; Iacopino, 2001).

Diabetes mellitus is a global public health problem and has a dramatic impact on the health care system not only due to high morbidity and mortality but also due to significant total medical costs (King, 2008). Smyth and Heron (2006) indicated that the prevalence of diabetes is significantly increasing annually by 2030. The World Health Organization (WHO) estimates there will be approximately 366 million people in the United States who have diabetes almost 6 million of these individuals are unaware that they have the disease, and therefore are unable to do what is needed to prevent long-term complications (Mealey and Oates, 2006).

The American Diabetes Association (ADA) classification of diabetes is based on the pathophysiology of each form of the disease, the two commonly encountered types of DM are type 1 (insulin -dependent diabetes) and type 2 (noninsulin-dependent diabetes) (ADA, 2003).

1.3.1 Diabetes MellitusType1

Type 1 diabetes, formerly insulin dependent diabetes or juvenile diabetes) is a form of DM that results from autoimmune destruction of insulin-producing beta cells of the pancreas. The subsequent lack of insulin leads to increased blood and urine glucose (Shoback *et al*., 2011). The classical symptoms of T1DM include: polyuria (frequent urination), polydipsia (increased thirst), xerostomia (dry mouth), polyphagia (increased hunger), fatigue, and weight loss. Type 1 diabetes are often first diagnosed when they present with diabetic ketoacidosis. These symptoms could be xeroderma (dry skin), rapid deep breathing, abdominal pain, and vomiting (Bluestone *et al*., 2010).

The rate of *B*-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainlyadults). The risk of a child developing T1DM is about 10% if the father has it, about 4% if the mother has T1DM and was aged 25 or younger when the child was born, and about 1% if the mother was over 25 years old when the child was born (Hana *et al*.,2006).

1.3.2 Diabetes Mellitus Type 2

Noninsulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes is a chronic disease characterized by high levels of sugar in the blood. T2DM develops when the body does not respond correctly to insulin (a hormone released by the pancreas). The most common form of diabetes is T2DM (Dastain *et al*., 2012).

This is in contrast toT1DM, in which there is an absolute insulin deficiency due to destruction of islet cells in the pancreas people with T2DM often have no symptoms at first. They may not have symptoms for many years (Shoback *et al*., 2011) .

Eisenbarth *et al*., (2011) indicated that early symptoms of T2DM may include: bladder, kidney, skin, or other infections that are more frequent or heal slowly, fatigue, hunger, increased thirst, increased urination.

Obesity is considered as one of the most significantly important risk factors of this disease (Passa, 2002; Sethi and Vidal-Puig, 2005).

Alternatively, some studies counted diabetes as one of the important metabolic complications of obesity. The role of obesity in the development of T2DM is now widely accepted (Trayhurn and Wood, 2004) . Complications with which it is associated, including of cardiovascular disease, including ischemic heart disease and increased rates of hospitalizations (Fasanmade *et al*., 2008). In the developed world, T2DM is the largest cause of nontraumatic blindness and kidney failure (Ripsin *et al*., 2009). It has also been associated with an increased risk of cognitive dysfunction and dementia disease processes such as Alzheimer's disease (Pasquier, 2010).

1.3.2.1 Pathophysiology of Type2 Diabete Mellitus

For many years, scientists have been trying to understand pathophysiology at the molecular level to control the elevated blood glucose in type 2 diabetes. (Imbeault *et al*., 2011).

The development of T2DM results from an interaction of a patient's genetic background with social and environmental factors of the nervous and a sedentary lifestyle, psychological stress, a high fat diet, over-eating, age, smoking and alcohol intake are some of the causes which pave the way to obesity, leading to diabetes (Grant *et al*., 2009). Alteration in communication between some vital organs such as the liver, skeletal muscles, pancreas, fat tissue, gastrointestinal tract and brain may result in an elevated blood glucose level, leading to T2DM. Since most patients with T2DM are overweight or obese, the role of fatty acid cannot be ignored in the development of diabetes (Martínez *et al*., 2010).

The correlation between obesity and insulin resistance may be assumed to be a “cause and effect” relationship, since clinical and preclinical studies indicate that weight loss/gain correlates closely with increasing/ decreasing insulin sensitivity (Vigneri *et al*., 2009).

Hyperglycemia has been claimed to be a prerequisite for lipotoxicity to occur and therefore the term glucolipotoxicity, rather than lipotoxicity, is more appropriate to describe the deleterious effects of lipids on β-cell function. The role of obesity in the pathophysiology of T2DM and insulin resistance has been a tested to in several studies (Eckardt *et al*., 2011).

The role of immune cells in promoting inflammation in obesity has been confirmed in humans. Obesity initially develops pro-inflammation starting from metabolic cells such as adipocyte and eventually recruits immune cells with the release of inflammatory cytokines such as TNF -α, IL-6 and adiponectin (Emanuela *et al.*,2012).

Secretion of leptin, TNF-α, resistin, adiponectin, inducible nitric oxide synthase (INOS) and an elevated plasma NEFA(non- esterified fatty acid) level gradually leads to obesity-induced inflammation that may interfere with glucose metabolism and insulin sensitivity and produce T2DM (Rabe *et al*., 2008).

Shinozaki *et al* (2011) indicated that enzyme INOS is a key inflammatory mediator in obesity and causes insulin resistance in the skeletal muscles. It inhibits secretion of adiponectin from adipocytes and impairs insulin secretion in the liver. Elevated INOS in the blood vessels causes vascular dysfunction in obesity.

Macrophage accumulation in adipose tissues in obese patients shares the expression of multiple genes causing adipose tissue inflammation in obesity. Similarly, some genetic modifications such as the glucokinase gene alter insulin secretion or function, leading to T2DM (Hellmann *et al*., 2011).

1.3.2.2 Association between periodontitis and Type2 Diabete Mellitus

There has been an increasing amount of literature on the relationship between periodontal disease and diabetes (Soskolne and Klinger, 2001). DM has long been reported to act as a risk factor for gingivitis and periodontitis (Preshaw *et al*., 2007).

The T2DM has been associated with higher prevalence, incidence and severity of periodontitis when compared with non-diabetic adults. In fact, the risk for periodontal disease is considerable in diabetes (both type 1 and 2) and it increases whenever the glycemic control is getting worse (Preshaw, 2008).

Several epidemiological studies have identified an increase in the extent and severity of periodontitis in diabetic adults (Bacic *et al*., 1988).

For instance, in a study of periodontal disease in Pima Indian of Arizona a population with a remarkably high prevalence of T2DM, researchers concluded that the prevalence and severity of attachment loss and bone loss was much greater among diabetic individuals when compared to non-diabetic controls in all age groups (Shlossman *et al*., 1990).

Therefore, it seems that glycemic control of diabetes is a pivotal factor contributing to the progression of periodontal disease. Several studies have confirmed that poor glycemic control promotes the development and progression of periodontitis (Peck *et al*., 2006).

In fact, data from the National Health and Nutrition Examination Survey (NHANES) were analysed to evaluate the relationship between glycemic control of T2DM and severe Periodontitis in US adult population ages 45 years and above. Of the 4343 persons in the NHANES III database, the poorly controlled diabetic subjects had a significantly greater prevalence of sever periodontitis when compared with non-diabetics on the contrary, diabetics with good glycemic control had no significant increase in the risk of periodontitis (Tsai *et al.,* 2002).

The evident deficiency of differences in periodontal pathogens between people with or without diabetes, suggests that alterations in the host immune

inflammatory response may be essentially responsible for the more aggressive periodontal destruction noted in patients with diabetes, it is clearly recognized that the immune-inflammatory response perform a pivotal role in the pathogenesis of periodontal disease. Periodontal disease occurrence depends on the interaction between microbial stimulation and the host response which seems to be orchestrated by a complex of cytokines working (Preshaw and Taylor, 2011). It has been demonstrated that the chronic inflammatory process contributes to the pathology associated with both diabetes and periodontal disease. It is also suggested that diabetes modifies periodontitis principally through its effect on the normal immune and inflammatory defenses (Southerl *et al*., 2006).

It is likely that modulations in inflammatory processes stemming from diabetes can give rise to a further dysregulation of the immune-inflammatory responses in the periodontium, resulting in increased periodontal destruction (Venza *et al.,* 2010).

There is growing evidence that diabetes is a state of chronic inflammation characterised by abnormal cytokine production For instance, elevated levels of IL-6 and TNF-α were recorded in plasma of obese patients and those with T2DM (Dandona *et al*., 2004). Hyperglycemia also results in elevated circulating concentrations of IL-6 and TNF-α (Esposito *et al*., 2002).

It has been shown that a close relationship presents among periodontitis, obesity and diabetes with adipose tissue and chronic inflammation being the common denominators (Genco *et al*., 2005).

Bastard *et al* (2006) indicated that adipose tissue may be deemed as a pivotal contributor in mechanism that links diabetes and periodontal disease. Several biologically active molecules are secreted by adipose tissue; these include cytokines such as IL-6 and TNF-α, and adipokines such as resistin, leptin and adiponectin. The cytokines have a direct proinflammatory effects on inflammatory cells, involving those in periodontal tissues. On the other hand, adipokines are important regulators of inflammatory responses (Sanchez-Margalet *et al*., 2003).

Furthermore, a variety of immune-competent cells are infiltrating adipose tissue such as macrophages and lymphocytes, which are responsible for an important part of the locally produced cytokines and adipokines (Weisberg *et al*., 2003).

1.4 Adipokines

Adipokines are bioactive mediators released from the adipose tissue including adipocytes and other cells present within fat tissues. These include several novel and highly active molecules released abundantly by adipocytes like leptin, resistin, adiponectin or visfatin, as well as some more classical cytokines released possibly by inflammatory cells infiltrating fat, like TNF-α, IL-6, IL-1ß (Tilg and Moschen., 2006).

The most abundantly expressed within the adipose tissue are leptin and adiponectin. Interestingly some reports indicate that adipocytes may also release the latter, more classical cytokines. Typical adipocytokines like leptin or adiponectin have been initially recognized through their role in the regulation of energy storage and homeostasis For example, leptin (Kaminski *et al.,* 2006). Plays an important role as a negative regulator of appetite control (Konturek *et al.,* 2004). Further research has shown that receptors for those proteins, are widely expressed, throughout the cardiovascular and immune system (Stallmeyer *et al.,* 2001).

The effects of adipocytokines on vascular function, immune regulation and adipocyte metabolism makes them key players in the pathogenesis of metabolic syndrome, a cluster of clinical symptoms including obesity, insulin resistance, hypertension, and dyslipidemia (Weiss *et al.,* 2004). Metabolic syndrome is one of the major risk factors of cardiovascular morbidity (Rabin *et al.,* 2005).

Release of adipocytokines may explain mechanisms of the relationship of obesity to cardiovascular phenotypes including hypertension, and atherosclerosis mainly through their ability to affect and modify endothelial and vascular function through their modulating effects on immune functions (Guzik *et al.*, 2003).

1.4.1 Adiponectin

A diponectin is a protein specifically secreted from white adipose tissue and also from the placenta in pregnancy in to the bloodstream and is very abundant in plasma relative to many hormones, adiponectin is a 244-amino-acid-long polypeptide (Chen , 2006) . There are four distinct regions of adiponectin. The first is a short signal sequence that targets the hormone for secretion outside the cell; next is a short region that varies between species; the third is a 65-amino acid region with similarity to collagenous proteins; the last is a globular domain. , when the 3-dimensional structure of the globular region was determined, a striking similarity to TNFα was observed (Shapiro and Scherer., 1998).

It circulates in two forms:

- A full length protein [High Molecular Weight (HMW) multimer and LowMolecular Weight (LMW) hexamer] as shown in figure (1.2). HMW form appears to be more active in insulin sensitivity and diabetes protection (Skurk, 2009)
- A proteolytic cleavage globular C-terminal domain which has Pharmacologic activity (Goldstein and Scalia., 2004). Adiponectin is a protein hormone that modulates a number of metabolic processes including decreased gluconeogenesis, increased glucose uptake, lipid catabolism, insulin sensevity, weight loss, control of energy metabolism (Vasseur *et al*., 2003).

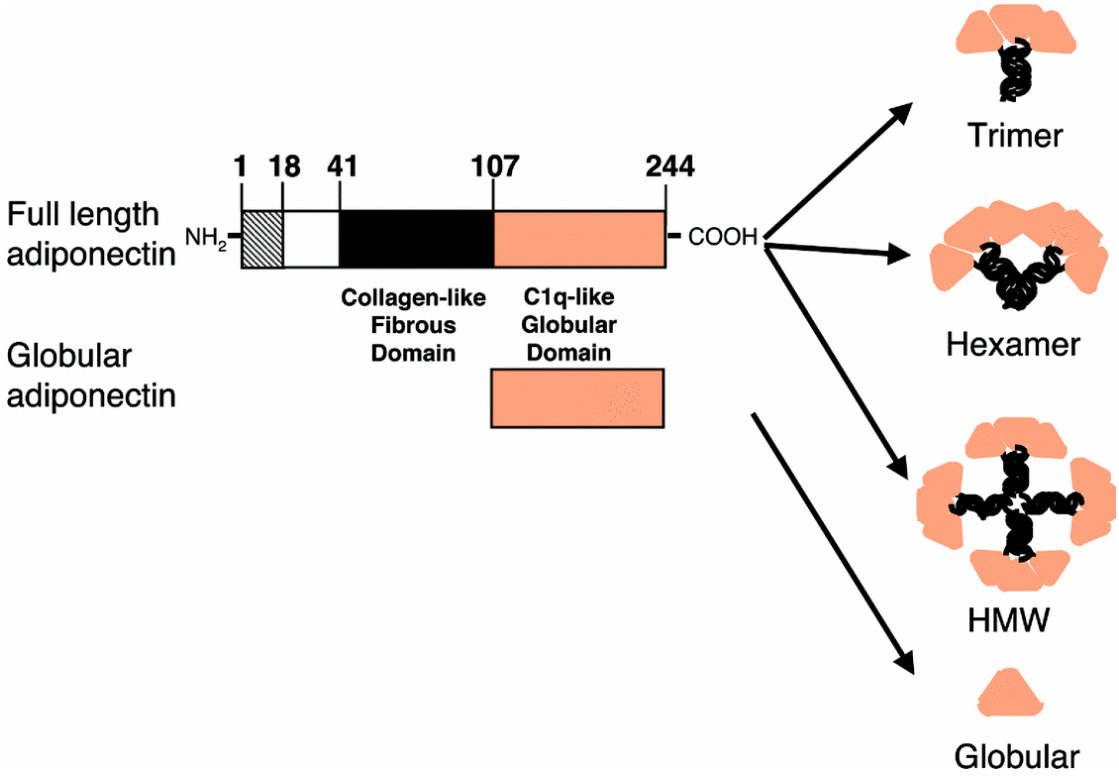


Figure (1.2): Molecular structure of Adiponectin (Skurk, 2009).

1.4.1.1 Adiponectin Receptors

Cell surface receptors for adiponectin have been cloned. They initiate signal transduction through phosphorylation and activation of AMP activated PK. which has pivotal role in regulating cholesterol synthesis, lipogenesis, lipid oxidation and glucose transport and oxidation. (Yamauchi *et al*., 2002).

- Two receptors were discovered: (Receptor 1) has higher affinity to globular adiponectin, while (Receptor2) has equal affinity to both globular adiponectin and full length adiponectin
- In skeletal muscles, (Receptor 1) is abundantly expressed, while in the liver (Receptor2) is more predominant. In the endothelium, both receptor types are expressed. Adiponectin receptors were also observed in adipocytes and macrophages (Tsuchida *et al*., 2004).

1.4.1.2 Correlation between Adiponectin in Periodontitis and Type2 Diabetes Mellitus

Serum adiponectin levels are decreased in obese humans and in patients with type 2 diabetes; hence, the A level tends to reflect insulin sensitivity. Adiponectin circulates in the blood in trimeric (low molecular weight, LMW), hexameric (medium molecular weight, MMW) and multimeric (high molecular weight, HMW) forms (Waki *et al*., 2003).

Among these forms, HMW adiponectin was shown to be decreased in obesity and diabetes as shown in the figure (1.3) and is associated with an increased risk for the development of diabetes, independent of total adiponectin (Heidemann *et al*., 2008). Recent studies suggested that the ratio of HMW to total adiponectin or to LMW and not the absolute amount of adiponectin in peripheral blood reflects insulin sensitivity. The adiponectin isoforms have different effects on human monocytes (Neumeier *et al*., 2006).

The globular form of adiponectin, which is generated from full-length adiponectin secreted from activated macrophages or neutrophils, is a powerful inducer of TNF-α and IL-6 secretion in primary human peripheral macrophages. However, pre-exposure of macrophages to globular adiponectin induced NF-κB activation and tolerance to further globular adiponectin and LPS exposure, resulting in the repression of TNF-α and IL-6 secretion (Tsatsanis *et al*.,2005). It has been reported that periodontal conditions did not relate to decreased adiponectin levels and the antimicrobial periodontal treatment did not increase adiponectin levels (Iwamoto *et al*., 2003) .This suggested that HMW adiponectin is proinflammmatory. The relationships of HMW adiponectin with periodontal inflammation should be examined (Yamaguchi *et al*., 2007).

Since the ratio of the high molecular weight of adiponectin to that of total may be a better predictor of periodontal inflammation Adipose TNF-α mRNA expression was elevated in obese mice and humans and the administration of adiponectin suppressed

TNF-α mRNA expression in local tissues and decreased circulating levels of TNF-α. The anti-inflammatory properties adiponectin appear to antagonize the effects of TNF-α (Kern *et al*., 2003).

This may be related to the structural resemblance between the cytokines, despite the lack of similarity between their primary sequences. Adiponectin has been shown to inhibit monocyte adhesion to endothelial cells and macrophage transformation to foam cells (Fasshauer *etal*., 2004).

It also appears to be important in bacterial and viral infections, it was shown to negatively regulate mouse macrophage-like cell responses to TLR ligands (Yamaguchi *et al*., 2005).Furthermore, it acted as an inhibitor of osteoclast formation stimulated by LPS from periodontopathic bacteria . Collectively, these observations suggest that adiponectin may inhibit alveolar bone loss in periodontitis (Karthikey and Pradeep., 2007).

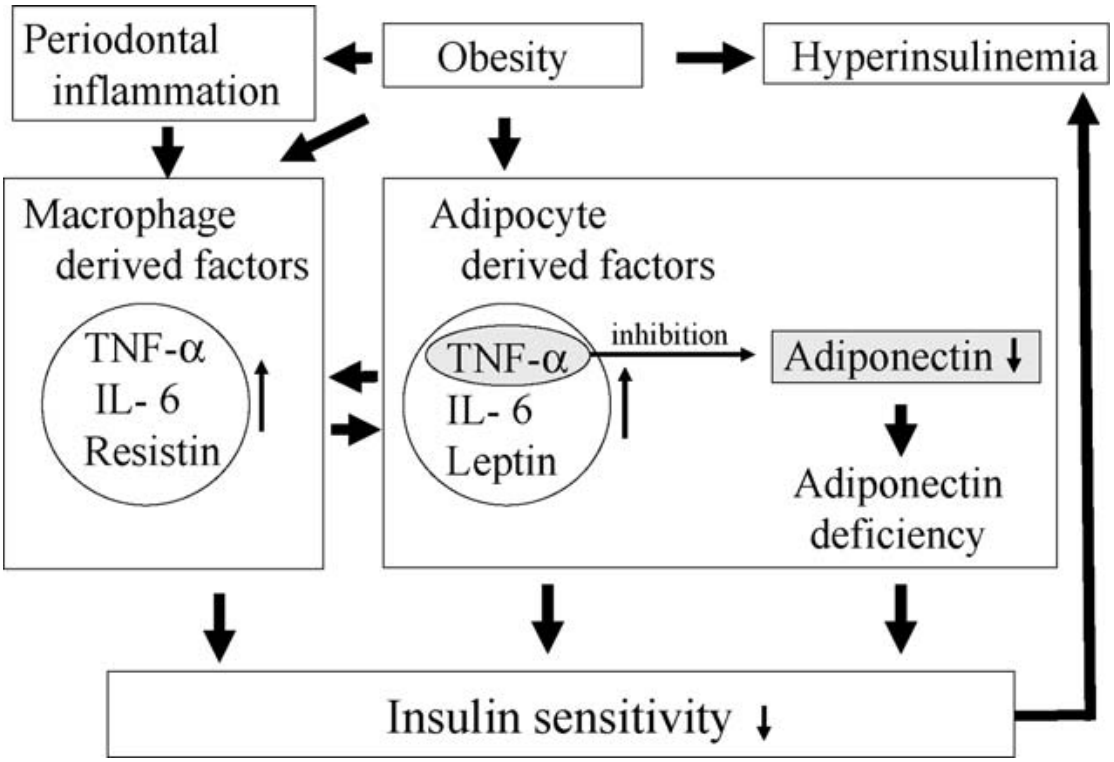


Figure (1.3): The relationship among periodontitis and adipokines (Kardesler *et al*., 2010).

1.4.2 Leptin

Leptin is a hormone made by fat tissue that acts on brain to regulate food intake and body weigh leptin is a 146 amino acid-long protein that is encoded by the obesity gene, the crystal structure of human leptin was found to have four helix bundle folds as shown in figure (1.4), closely resembling the structures of other class I helical cytokines, such as IL-2 , IL-12 . So it considered to be a member of the class I cytokine superfamily (Huising *et al*., 2006)

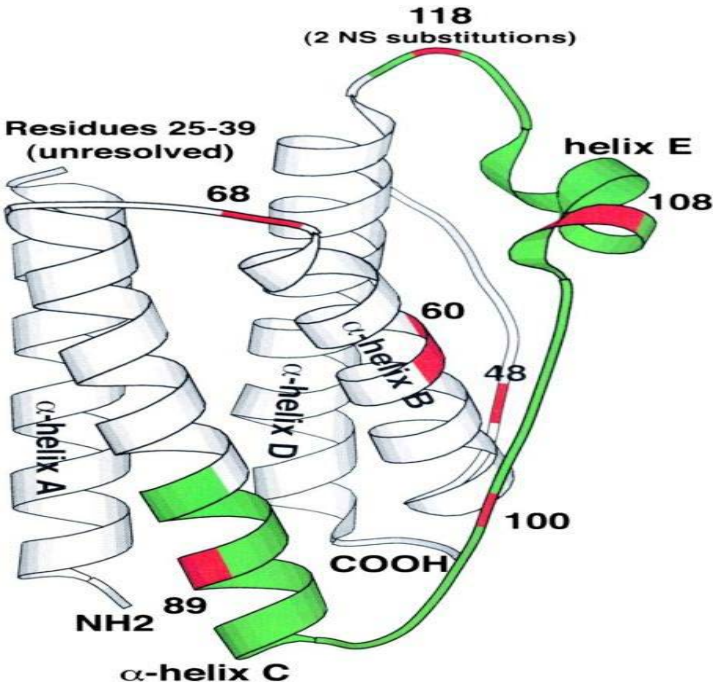


Figure (1.4): Chemical structure of human leptin (Zhang *et al*., 1997).

It is manufactured primarily in the adipocytes of white adipose tissue and the level of circulating leptin is proportional to the total amount of fat in the body. In addition to white adipose tissue—the major source of leptin it can also be produced by brown adipose tissue, placenta ovaries, skeletal muscle, bone marrow, pituitary, and liver (Margetic *et al*., 2002). This hormone circulates in blood and acts on the hypothalamus to regulate food intake and energy expenditure. When fat mass falls, plasma leptin levels fall stimulating appetite and suppressing energy expenditure until fat mass is restored. When fat mass increases, leptin levels increase, suppressing

appetite until weight is lost, this physiological system ensures that total energy stores are stably maintained within a relatively narrow range (Deloumeaux *et al*., 2011).

Leptin acts on receptors in the hypothalamus of the brain, where it inhibits appetite counteracting the effects of neuropeptide Y (a potent feeding stimulant secreted by cells in the gut and in the hypothalamus) (Pratley *et al*., 1997).

The absence of leptin leads to uncontrolled food intake and resulting obesity. Several studies hav shown fasting or following a very-low-calorie diet (VLCD) lowers leptin levels (Weigle *et al*.,1997). In the short-term, leptin might be an indicator of energy balance. This system is more sensitive to starvation than to overfeeding; leptin levels change more when food intake decreases than when it increases (Chin-Chance *et al*., 2000).

1.4.2.1 Leptin receptors (ObR)

The leptin receptor is a member of the class I cytokine family of cytokine receptors, since they all share a similar three-dimensional folded structure and activate similar signalling pathways (Huising *et al*., 2006). This group of receptors includes various members of the interleukin receptor family. Leptin receptors are detected approximately on all cell types, organs and tissues, leptin receptors are expressed on the hypothalamus, cerebellum, blood brain barrier, salivary glands (Bohlender *et al*., 2003), adipocytes, intervertebral discs, skeletal muscle and osteoblast (Reseland *et al*., 2001; Guerra *et al*., 2007).

In the immune system, leptin receptors are expressed on T cells, natural killer cells, neutrophils, dendritic cells, monocytes and lymphoid tissues (Siegmund *et al*., 2004).

1.4.2.2 Leptin in periodontitis and Type 2 Diabetes Mellitus

It is well known that leptin synthesis is increased by a number of inflammatory stimuli, including IL-1, IL-6, TNF-α, and LPS (Sarraf *et al*.,1997).

An increase in leptin secretion during infection inflammation strongly suggests that it is involved in the cytokine network that governs host defense mechanisms.

Leptin receptors are known to be expressed in adipocytes, T lymphocytes, and vascular endothelial cells (Lei *et al*., 2004).

Karthikey and Pradeep reported that leptin concentrations in gingival crevicular fluid (GCF) were found to be higher in healthy gingiva than in tissues with periodontitis. Conversely, serum Leptin levels are increased in people with periodontitis (Karthikey and Pradeep, 2007).

In a recent study, elevated serum leptin concentration is associated with chronic periodontitis and acute myocardial infarction and could be considered as one of the risk markers for the diseases (Gundala *et al.*, 2012). However, other study has reported that gingival tissues contain leptin receptor . The serum levels of leptin do not vary between patients and with different periodontal conditions (Ayz *et al.*, 2012).

A large body of evidence indicates that leptin along with insulin exert an inhibitory effect on food intake, and an activation effect on the regulation of thermogenesis within the central nervous system (Dhillon *et al*., 2001).

Leptin and insulin function as a critical signal to the brain in the long-term regulation of energy homeostasis, the exact relationship between leptin and insulin is not clear and is sometimes controversial (Emilsson *et al*., 1996).

Although insulin is secreted from pancreatic beta cells rather than from adipocytes, the secretion of both hormones is influenced by the overall amount of fat stores as well as by short-term changes in energy balance (Chen *et al*., 1999).

Moreover, insulin receptors are located in the same key hypothalamic areas as leptin receptors. Whereas insulin secretion is stimulated actualley in response to meals, while leptin secretion is not (Ookuma *et al*., 1998). Although the mechanisms governing leptin secretion have yet to be fully elucidated, insulin appears to play a key role most obese mammals have elevated plasma concentrations of leptin and the insulin they appear to be resistant to leptin induced anorexia (Ceddia *et al*., 2002).Therefore the relationship between these two hormones should be revealed through the metabolic effects of these hormones on energy balance (Segal *et al*., 1996).

Both T2DM and obesity are associated with poorer cognitive function. Potential mechanisms include raised circulating inflammatory markers and cortisol level (Ripsin *et al*., 2009). Which have been linked to poorer cognitive performance in people withT2DM, The role of other hormones, such as leptin, levels of which are increased in subjects with obesity and T2DM, has not been investigated (Morrison, 2009).

Chapter Two

Materials and Methods

Materials and Methods

2.1. Subject

2.1.1 Patients Group

A total of 60 patients with periodontitis (30 patients with T2DM and 30 patients without T2DM) were included in this study, their age range (32 – 64) years. They were from attendants seeking treatment in the department of periodontics, College of Dentistry, Baghdad University from October 2013 to December 2013. Diagnosis was made by specialized dentists in the College. Patients with DMT2 were selected on the basis of criteria for diabetes were used according to the American Diabetes Association guideline; fasting plasma glucose up to 7 mmol/l and above was considered to be diabetic and levels between 5.55 mmol/l and 6.9 mmol/l was considered as impaired fasting glucose (ADA,2003).

The diagnosis of patients with T2DM was made by special physician in the Baghdad teaching hospital and the result of FBG test for periodontitis patients with T2DM obtained from the Emergency laboratory in the Baghdad teaching hospital and the FBG levels ranged between (114-295 mg/dl). The exclusion criteria included Individuals with cardiovascular disease, renal failure, rheumatoid arthritis, other systemic diseases and smoker individuals.

The patients were subjected for a questionnaire about (age, gender, BMI, family history of diseases, presence of diabetes, period of infection and smoking) as shown in (appendix I ,II).

2.1.2 Control Group

Apparently healthy volunteers their ages and gender were matched with patients, consisted of 25 individuals who were considered as control (8 males and 17 females), their age ranged between (32-64) years. All of them had no sign of periodontitis or T2DM and they were free of any medical complications.

2.2 Materials

2.2.1 Equipments & Apparatus

The equipments and apparatus used throughout this study are mentioned in Table (2-1).

Table 2-1: The equipments and apparatus used throughout the study.

Equipment and Apparatus	Company	Origin
Disposable syringes	CMP	Turkey
Test tubes	Grenier	Germany
Centrifuge	G.I.G	U.S.A
Eppendorff tubes	Eppendroff	Germany
Disposable micropipette tip (yellow & blue)	Walter	Germany
Micropippte	Slamed	Germany
Refrigerator	Fenos	Turkey
Water bath	Grant	England
Vortex mixer	Frost	England
ELISA Incubator	Pasteur	France
ELISA Washer	Human	Germany
ELISA Reader	BioRAD	Australia
ELISA Printer	Epson LX-300	Italy

2.2.2 Kits

Table 2-2: Kits used through the study

KITS	Company	Country
1.Adiponectin	Cusabio	China
2.Leptin	Cusabio	China

2.3. Methods

2.3.1 Serum samples treatment

Approximately 5 ml of fasting human blood was collected from each subject (patient and control) and transferred into sterilized test tubes and allowed for 30 minute to clot at room temperature , the sample was centrifuged for 5 minutes at 3500 rotations per minute and the serum was immediately separated and stored at (- 20 ^{0} C) till used for adiponectin and leptin hormone analysis.

2.3.2 Body Mass Index (Quetelet index)

Body mass index was calculated by the ratio of weight to the square of the height measured in kilograms/metres^{2}. Normal weight is defined as a BMI of 18.50-24.99, overweight as between 25 and 29.99 and obesity as greater or equal to 30 BMI concentrates on an individual's height and weight to determine and estimated body fat level (Stommel and Schoenborn, 2009) and according to the table shown in (Appendix III).

2.3.3. The Clinical Periodontal Parameters

Oral examination was performed by the same examiner. The collected data include:-

2.3.3.1 Plaque Index (PI)

The four surfaces of each tooth except 3rd molar were examined and scored according to the plaque index system (Silness and Loe, 1964).

2.3.3.2 Gingival index (GI)

The occurrence of gingival inflammation at four surfaces of each tooth except 3rd molar was assessed using the criteria of the GI by Loe and Sillness (1963).

2.3.3.3 Probing Pocket Depth measurement (PPD)

The probing pocket depth is defined as the distance from the gingival margin to the base of the pocket; it was measured by using a calibrated periodontal probe, no pressure was used, the probe was allowed to fall by its own weight (Carranza, 2009).

2.3.3.4 Assessment of clinical attachment level (CAL)

Clinical attachment level was defined as the distance from the cemento-enamel junction (C.E.J) to the base of the pocket with the use of the calibrated periodontal probe. In some instances the gingival cover the C.E.J., so the CAL was assessed by subtracting the distance from the gingival margin to C.E.J. from the pocket depth **(**Loe and Brown, 1991).

2.3.3.5. Bleeding on probing (BOP)

The periodontal probe inserted in to the bottom of the periodontal pocket .If bleeding is provoked by this instrument within 30 seconds after probing, the site was given positive score and a negative score for non- bleeding site (Salvi *et al*., 2008).

2.4. Detection of Serum Adiponectin (Pauli, 2004).

2.4.1. Principle

This assay employs the quantitative sandwich enzyme immunoassay technique .The microtiter plate provided in this kit has been pre-coated with antibody specific to adiponectin. Standards or samples are then added to the appropriate microtiter plate wells and any adiponectin present is bound by the immobilized antibodies . After removing any unbound substance, a biotin-conjugated antibody specific for adiponectin is added to the wells, after washing avidin conjugated horseradish peroxidase (HRP) is added to each microplate well and incubated. Following a wash to remove any unbound avidin- enzyme reagent.

3,3'5, 5' tetramethyl-benzidine (TMB) a substrate solution is added to each well. Only those wells that contain adiponectin, biotin-conjugated antibody and enzyme-conjugated avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm . The concentration of adiponectin in the samples is then determined by comparing the optical density (OD) of the samples to the standard curve.

2.4.2.Materials provided with kit

Table 2-3: Materials provided with Adiponectin kit

Reagent	Quantity
Assay plate(12x8 coated microwells)	1(96 well)
Standard	2
Sample Diluent	1x50 ml
Biotin-antibody Diluent	1x15ml
Biotin-antibody(100xconcentrate)	1x20µl
HRP-avidin Diluent	1x15ml
HRP-avidin(100xconcentrate)	1x20µl
TMB Substrate	1x10ml
Wash Buffer(25xconcentrate)	1x20ml
Stop Solution	1x10ml
Adhesive strip(for 96 wells)	4
Instruction mnual	1

2.4.3. Reagent preparation

All reagents were brought to room temperature (18-25°c) before prepared and used for 30 min.

1.Wash Buffer: was mixed gently and then diluted 20 ml of wash buffer concentrate with distilled water to prepare 500 ml of wash buffer.

2. Standard : vial was centrifuged at 1000-6000 rpm for 30 second, then added 1ml of sample diluent. This reconstitution produced a stock solution of 100ng/ml, the stander was mixed to ensure completely reconstitution then allowed the stander to site for 10 minutes ,then pipette 250 µl of sample diluent into each tube (s0 -s6),the stock solution was used to produce a 2-fold dilution series. Each tube was mixed thoroughly before the next transfer. The undiluted standard serves as the high standard (100 ng/ml) and sample diluent serves as the zero standard (0ng/ml).

3. Biotin-antibody: the vial was centrifuged at 3000 rpm for 30 second before opening in the sterilized test tube added (11,880 µl of biotin-antibody diluent + 120 µl of biotin antibody).

4. HRP-avidin**:** the vial was centrifuged at 3000 rpm for 30 second before opening in the sterilized test tube added (11,880 µl of HRP- avidin diluent + 120 µl of HRP-avidin).

5.Sample preparation: all the samples ejected from the refrigerator and thawed by putting in the water bath at 37°c for 20 minute then diluted serum sample with sample diluent (1:500) before the test. The 500-fold dilution achieved by adding 5 µl of sample to 95 µl of sample diluent first, then completed the 500-fold dilution by adding 10 µl of this solution to 240 µl of sample diluent

2.4.4. Test Procedure

1. One hundred micro litter of standard and sample were added per well and covered with the adhesive strip then Incubated in Incubator for 2 hours at 37°c.

2. The liquid was removed from each well without washing.
3. One hundred µl of biotin- antibody was added to each well, then covered with a new adhesive strip and incubated for 1 hour at 37°c.
4. Each well was aspirated and washed by using ELISA washer which filled with wash buffer , this process was repeated for three times for a total of three washes.
5. One hundred micro-litter of HRP- avidin was added to each well then covered with a new adhesive strip and incubated for 1 hour at 37°c.
6. The aspiration and wash process were repeated for five times as step 4.
7. Ninety µl of TMB Substrate was added to each well. TMB substrate changed from colorless to blue color, then covered with a new adhesive strip and incubated for 30 minutes at 37°c. The plate was protected away from light.
8. Fifty µl of stop solution was added to each well .The color was turned from blue to yellow.
9. The optical density of each well was determined within 30 minutes, by using a microplate ELISA reader set to 450 nm.

2.4.5 Interpretation of results

As the assay was completed, the absorbance of each sample and standard was calculated and this calculation usually appears on the result sheet obtained by ELISA printer.

The results were analyzed automatically by creating a standard curve by inserting the data using computer software capable of generating four parameter logistic curve-fit . Stander curve constructed by plotting the mean absorbance for each standard and sample on the x-axis against the concentration of stander on the y-axis and draught a best fit curve through the points on the graph. The data may be linearized by plotting the log of the adiponectin concentrations versus the log of the OD and the best fit line can be determined by regression analysis. The samples had been diluted, the concentration reads from the standard curve multiplied by the dilution factor.

2.5 Detection of Serum Leptin (Keiichi *et al*., 1998)

2.5.1 Principle

This assay employs the quantitative sandwich enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with antibody specific to leptin. Standerds or samples are then added to the appropriate microtiter plate wells and any leptin present is bound by the immobilized antibody. After removing any unbound substance, a biotin-conjugated antibody specific for leptin is added to the wells , after washing avidin conjugated (HRP) is added to each microplate well and incubated. Following a wash to remove any unbound avidin-enzyme reagent.

3,3'5, 5' TMB a substrate solution is added to each well. Only those wells that contain leptin, biotin-conjugated antibody and enzyme-conjugated avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometry at a wavelength of 450 nm . The concentration of leptin in the samples is then determined by comparing the OD of the samples to the standard curve.

2.5.2 Materials provided with kit

Table 2-4: Materials provided with Leptin kit

Reagent	Quantity
Assay plate(12x8 coated microwells)	1(96 well)
Standard	2
Sample Diluent	1x50 ml
Biotin-antibody Diluent	1x15ml
Biotin-antibody(100xconcentrate)	1x20µl
HRP-avidin Diluent	1x15ml
HRP-avidin(100xconcentrate)	1x20µl
TMB Substrate	1x10ml

Wash Buffer(25xconcentrate)	1x20ml
Stop Solution	1x10ml
Adhesive strip(for 96 wells)	4
Instruction mnual	1

2.5.3 Reagent preparation

All reagents were brought to room temperature (18-25°c) before prepared and used for 30 min.

1. Wash Buffer: was mixed gently and then diluted 20 ml of wash buffer concentrate into distilled water to prepare 500 ml of wash buffer.
2. Standard : vial was centrifuged at 1000-6000 rpm for 30second.then added 1ml of sample diluent .This reconstitution produced a stock solution of 10ng/ml.

   The stander was mixed to ensure completely reconstitution then allowed the stander to site for 10 minutes, then pipette 250 µl of sample diluent into each tube (s0 - s6).The stock solution was used to produce a 2-fold dilution series. Each tube was mixed thoroughly before the next transfer. The undiluted standard serves as the high standard (10 ng /ml) and sample diluent serves as the zero standard (0ng/ml).
3. Biotin-antibody : the vial was centrifuged at 3000 rpm for 30 second before opening in the sterilized test tube added (11,880 µl of biotin-antibody diluent + 120 µl of biotin antibody).
4. HRP-avidin: the vial was centrifuged at 3000 rpm for 30 second before opening in the sterilized test tube added (11,880 µl of HRP- avidin diluent + 120 µl of HRP-avidin).
5. Sample preparation: all the samples ejected from the refrigerator and thawed by putting in the water bath at 37 °c for 20 minute then diluted serum sample with sample diluent (1:500) before test. The 500-fold dilution achieved by adding 5 µl of sample to 95 µl of sample diluent first, then completed the 500-fold dilution by adding 10 µl of this solution to 240 µl of sample diluent

2.4.4. Test Procedure

1. One hundred µl of standerd and sample were added per well and covered with the adhesive strip then incubated in incubator for 2 hours at 37°c.
2. The liquid was removed from each well without washing.
3. One hundred µl of biotin- antibody was added to each well, then covered with a new adhesive strip and incubated for 1 hour at 37°c.
4. Each well was aspirated and washed by using ELISA washer which filled with wash buffer , this process was repeated for three times for a total of three washes.
5. One hundred µl of HRP- avidin was added to each well, then covered with a new adhesive strip and incubated for 1 hour at 37°c.
6. The aspiration and wash process were repeated for five times as step 4.
7. Ninety µl of TMB substrate was added to each well. TMB substrate changed from colorless to blue color, then covered with a new adhesive strip and incubated for 30 minutes at 37 °c. The plate was protected away from light.
8. Fifty µl of stop solution was added to each well, the color was turned from blue to yellow.
9. The OD of each well was determined within 30 minutes, by using a microplate ELISA reader setted to 450 nm.

2.5.5 Interpretation of results

As the assay was completed, the absorbance of each sample and stander was calculated and this calculation usually appears on the result sheet obtained by ELISA printer.

The results were analyzed automatically by creating a standard curve by inserting the data using computer software capable of generating four parameter logistic curve-fit .

Stander curve constructed by plotting the mean absorbance for each standard and sample on the x-axis against the concentration of stander on the y-axis and draught a best fit curve through the points on the graph.

The data may be linearized by plotting the log of the leptin concentrations versus the log of the OD and the best fit line can be determined by regression analysis. The samples had been diluted, the concentration reads from the standard curve multiplied by the dilution factor.

2.6. Statistical Analysis

Data analysis was computer assisted using SPSS version 19 computer program (statistical package for social science). The statistical analyses included; descriptive statistics (medians, means, standard deviations, minimum and maximum values) and inferential statistics (T-test to, ANOVA test and Spearman's rank correlation coefficient).

Frequency distribution for selected variable was done first, the statistical significance of different in mean of quantitative normally distributed variable between 2 group was tested by independent sample t-test whereas among three groups were tested by ANOVA test.

P value less than the 0.05 level of significance was consider statistically significant.

The Spearman's correlation coefficient test was used to test the relation between leptin and adiponectin in each group and their relation with the clinical periodontal parameters.

Chapter Three

Results and Discussion

Results and Discussion

3.1 Distribution Features and Clinical Parameters

3.1.1 Distribution of healthy control and patients according to age, gender and BMI

The results presented in this study are based on the analysis of 60 periodontitis patients consist of 30 patients without T2DM and 30 patients with T2DM, compared with 25 healthy individuals considered as controls.

The characteristics of patients groups and control group included in this study are presented in table (3.1). No statistically significant differences ($p>0.05$) in age or gender existed between study groups. The mean age of periodontitis patients was 47.60±8.48 years and the mean age of periodontitis patients with T2DM was 48.16±8.34 years, while in control group was 44.76±8.29 years. Furthermore, there was a slight male's peredominance among periodontitis group about (63.3%) of patients were males, while only (36.7%) were females, in periodontitis +T2DM group about (60%) of patients were males, and (40%) were females. A predominance of periodontitis in males more than a female in this study which is comparable with other Iraqi study conducted by Mohssen (2013), whereas, disagree with the result of (Ali *et al.*, 2008) who observed that periodontitis in males less than as in females. The large prevalence of periodontitis among males may be due to that male less interest in oral hygiene than females.

Regarding the mean of BMI, the current results found that there are no significant differences ($p>0.05$) in the mean of BMI among study groups; in periodontitis group the mean of BMI was 22.43±4.10 Kg/m2, in periodontitis +T2DM group the mean of BMI was 22.95±3.4 Kg/m2 and in control group the mean of BMI was 22.42±3.25 Kg/m2, as clearly shown in table (3.1).

From the body weight and square of the height, BMI was calculated for each of the healthy individuals and two groups of patients. Results declared that all the patients and control groups can be classified to normal weight (18.50 – 25 kg /m^2). Current result was comparable with other local study performed by Karam (2013) who found that there is no siginificant difference in the mean BMI between periodontitis patients and control group.

Table 3.1: Distribution of ages, sexes and BMI in study groups.

Characteristics		Study groups			P-value
		Healthy control n=25	Periodontitis n=30	Periodontitis +T2DM n=30	
Age and Sex					
Age (years)	Range	(32-64)	(32-64)	(32-64)	
	Mean ± SD	44.76±8.29	47.60±8.48	48.16±8.34	0.43^{NS}
Gender type	Female	17 (68%)	11(36.7%)	12 (40%)	
	Male	8 (32%)	19 (63.6 %)	18 (60%)	0.672^{NS}
BMI (Kg/m2)					
BMI	Mean ± SD	22.42±3.25	22.43±4.10	22.95±3.4	0.665^{NS}

NS=Not significant (p>0.05).

3.1.2 Clinical Periodontal Parameters in Patients and Control

The values of clinical periodontal parameters in patients groups were significantly different from that in healthy controls. Table (3.2) and figure (3.1) demonstrated that the mean value of PI, GI, PPD, CAL and BOP were significantly higher (P<0.001) in periodontitis group (1.43±0.39; 1.30±0.46; 2.23±0.79; 2.37±0.85 and 25.34±26.61) and in periodontitis patients with T2DM (1.54±0.66; 1.29±0.45; 2.23±0.94; 2.30±1.03 and 24.66±9.79) when compared to controls group (0.79±0.39; 0.74±0.28; 0.84±0.39; 0.0; 5.76±1,67), respectively. On the other hand, there are no significant differences (p>0.05) in the the mean value of all clinical periodontal

parameters (PI, GI, PPD, CAL and BOP) between two groups of periodontitis patients.

The present result was consistent with other result reported by Abaas (2006) and AL-saidy(1996), they found that there was significant increase in mean of periodontal parameters (PI ,GI, PPD, CAL and BOP) in patients with T2DM compared to healthy control. Similarly (Karam, 2013) found that the mean value of each PI, GI, PPD, CAL and BOP were significantly higher in periodontitis patients when compared to healthy controls.

Although the bacterial biofilm is necessary for the development of the periodontal disease, it alone is not enough to produce the disease. The host response, through the releasing of a large spectrum of proinflammatory mediators, is responsible for great part of the periodontal tissue destruction observed in the disease (Andriankaja *et al*., 2010).

Several other factors possibly contribute to the development of the periodontal disease. Obesity, hypertension , dyslipidemia and insulin resistance or diabetes – components of the metabolic syndrome – has been suggested as risk factors for periodontal disease (Odili and Staessen , 2010) .

There is substantial evidence from cross-sectional and prospective studies that people with types 1 and 2 diabetes have more than double the risk of developing periodontitis (Firati, 1997).

Rodrigues *et al*.,(2003), Indicated that diabetes can also result in more severe periodontal destruction than in matched non-diabetes groups. However, increase the risk for periodontitis is dependent on glycaemic control and the duration of diabetes.

Table 3.2: Clinical Periodontal Parameters in Study Groups.

Clinical periodontal Parameters	Study groups			P-value
	Healthy control n=25	Periodontitis n=30	Periodontitis+ T2DM n=30	
Plaque index	0.79±0.39	1.43±0.39	1.54±0.66	<0.001**
Gingival Index	0.74±0.28	1.30±0.46	1.29±0.45	<0.001**
Probing Pocket Depth (mm)	0.84±0.39	2.23±0.79	2.23±0.94	<0.001**
Clinical Attachment Loss (mm)	0.0	2.37±0.85	2.30±1.03	<0.001**
Bleeding on Probing (BOP)	5.76±1.67	25.34±26.61	24.66±9.79	<0.001**
Control X periodontitis<0.001 **Control X periodontitis +T2DM<0.001** **periodontitis X periodontitis +T2DM** [NS]				

** = Highly significant difference (p≤0.001).

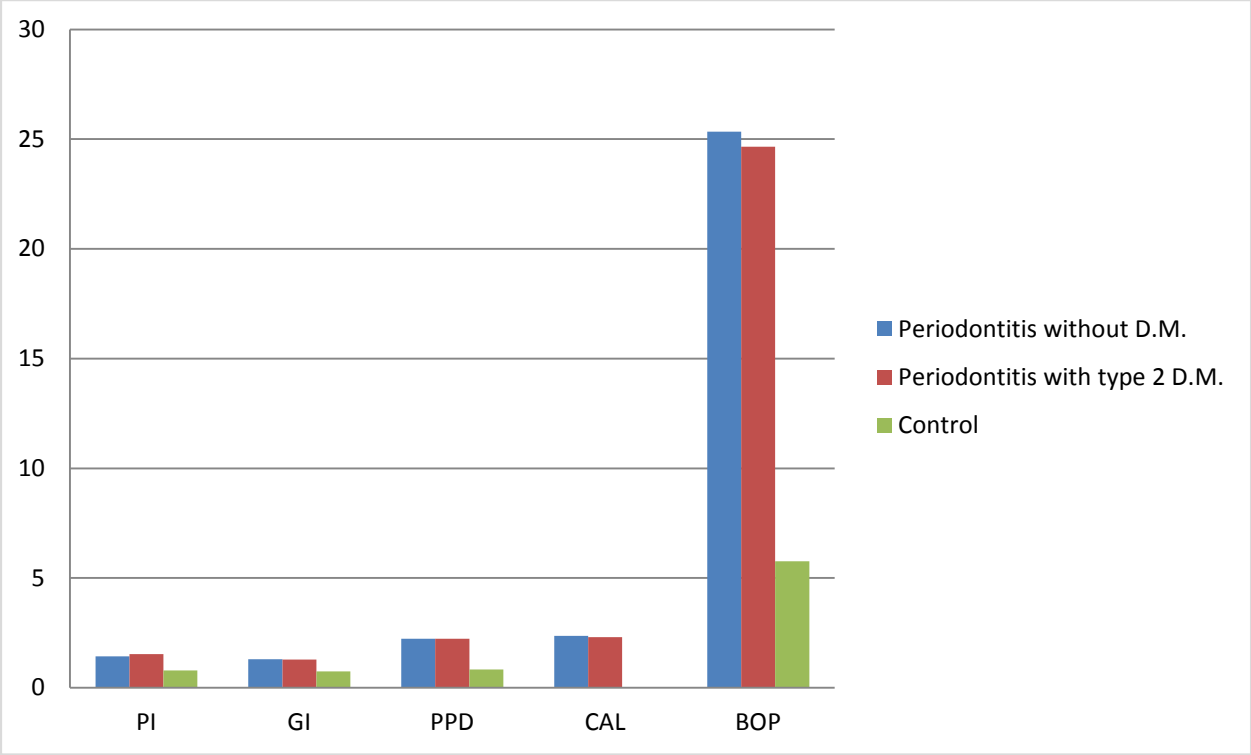


Figure 3.1: Clinical Periodontal Parameters in Study Groups.

3.2 Serum levels of Leptin

Table (3.3) and figure (3.2) revealed a significant elevation in mean serum level of leptin in periodontitis group (25.89±5.52 ng/ml) and in periodontitis+T2DM patients (32.16±7.78 ng/ml) as compared to healthy control (16.66±3.93 ng/ml), ($p<0.01$). Moreover, the comparison between two groups of patients showed that the mean level of leptin was increase in periodontitis+T2DM group but statistically not significantly ($p>0.05$). The results was similar to the study performed by Karam (2013), who showed that the circulating level of leptin in serum was correlated positively with periodontitis diseases. Correspondingly, Karthikey and Pradeep in (2007), who suggest that greater the periodontal destruction, the greater in the serum leptin concentration and the lowest serum leptin concentration was found in healthy individuals. In addation, this result agreed with study performed by Turki *et al* in (2012), who observed a significant increased level of leptin in type 2 diabetic male patients.

Wu *et al* in (2000), who demonstrated that leptin levels in diabetics are higher than in normal subjects and that T2DM is associated with hyperinsulinaemia and insulin resistance compared to the control group.

Two explanations have been proposed for the increase of the serum levels of leptin in periodontitis: firstly, the gingival inflammation would result in vasodilatation, which would increase the serum levels of leptin. Secondly, the serum levels of leptin would increase as a defense mechanism of the body, to fight the periodontal inflammation (Bullon *et al.*,2009). Recently, Gundala *et al* in (2012) mentioned that elevated serum leptin concentration is associated with chronic periodontitis could be considered as one of the risk markers, and Duarte *et al* in (2012) found that serum level of leptin was significantly higher in periodontitis patients when compared to healthy controls suggesting that periodontitis upregulated the circulating level of leptin in subjects with normal BMI. In contrast to the present result Davies *et al* in (2011) pointed out to that the level of serum leptin was not significantly different between periodontitis patients and healthy subjects.

Table 3.3: The differences in mean serum levels of leptin (ng/ml) among study groups.

Serum Leptin	Control group n=25	Periodontitis n=30	Periodontitis+T2DM n=30	p-value
Range	(1.10-50.92)	(0.7-77.55)	(1.84-60.14)	
Median	11.0	23.93	22.82	
Mean	16.66	25.89	32.16	**0<0.01***
S.D.	3.93	5.52	7.78	
Control Vs periodontitis0<0.01* Control Vs periodontitis +T2DM 0<0.01* Periodontitis Vs periodontitis +T2DM >0.05				

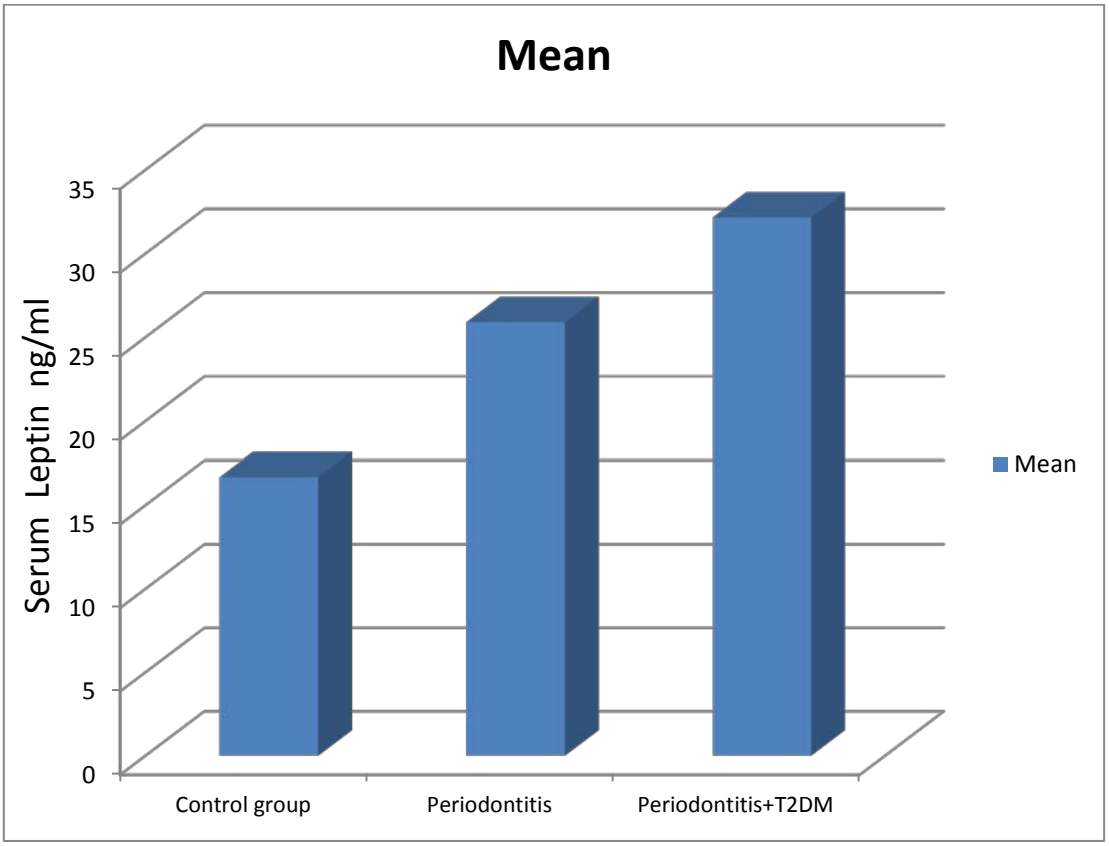


Figure 3.2: The differences in mean serum levels of leptin among study groups.

3.3 Serum levels of Adiponectin

The current study observed that there is significant decrease ($p<0.001$) in mean serum level of adiponectin in periodontitis patients (60.08±9.61 ng/ml) and periodontitis+T2DM patients (50.10± 7.64 ng/ml) in comparsion to that in healthy control (77.57±10.80 ng/ml). Additionally, there is slight significant reduction in mean serum level of adiponectin in among periodontitis patients with T2DM when compared to those patients without T2DM, ($p<0.05$), according to table (3.4) and figure (3.3).

Adiponectin enhances production of anti-inflammatory cytokines including IL-10 and the IL-1 receptor antagonist, part of the evidence for its anti-inflammatory role. Furthermore, adiponectin can indirectly decrease IL-6 and TNF-α. Because of its anti-inflammatory properties, adiponectin is important in metabolic disorders including obesity, type II diabetes, coronary heart disease, and metabolic syndrome (Oda *et al*., 2008).

The present results agreed with study performed by (Jing Ling *et al*., 2014), who indicated that decresed levels of serum adiponectin in patients of (periodontitis+T2DM diseases) and periodontitis patients when compared to healthy control. Prospective and longitudinal studies indicated that lower adiponectin levels were associated with a higher incidence of type 2 diabetes (Mather *et al.,* 2008). Pischon *et at.,* 2007, reported that adiponectin is a hormones secreted from the fat tissue, the levels of adiponectin are reduced in people with obesity, insulin resistance and T2DM. Moreover, another study done by Luo *et al* in (2010), demonstrated that lower adiponectin level associates with impaired gloucose tolerance and T2DM in Asian Indian women.On the other hand, the results reported by Furugen *et al* in (2008) were at variance with current results, who indicated that there was no significant differences in adiponectin levels among patients with periodontitis in Japanese people when compared to healthy controls.

Moreover Wei-Lian *et al* .(2010), recommended that reduced serum adiponectin and increased inflammatory cytokines in patients with periodontitis and

T2DM,at 3 months after periodontal treatment is helpful for glucose control, which lead to increased serum adiponectin levels and reduced inflammatory cytokine levels.

Table 3.4: The differences in mean serum levels of adiponectin (ng/ml) among study groups.

Serum Adiponectin	Healthy control (25)	Periodontitis (30)	Periodontitis + T2DM (30)	p-value
Range	(17.72-94.44)	(19.24-94.36)	(21.05-98.44)	
Median	79.22	63.40	63.40	
Mean	77.57	60.08	50.10	0<0.001**
SD	10.80	9.61	7.64	
Control Vs periodontitis<0.001** Control Vs periodontitis+T2DM <0.001** Periodontitis Vs periodontitis+T2DM <0.05*				

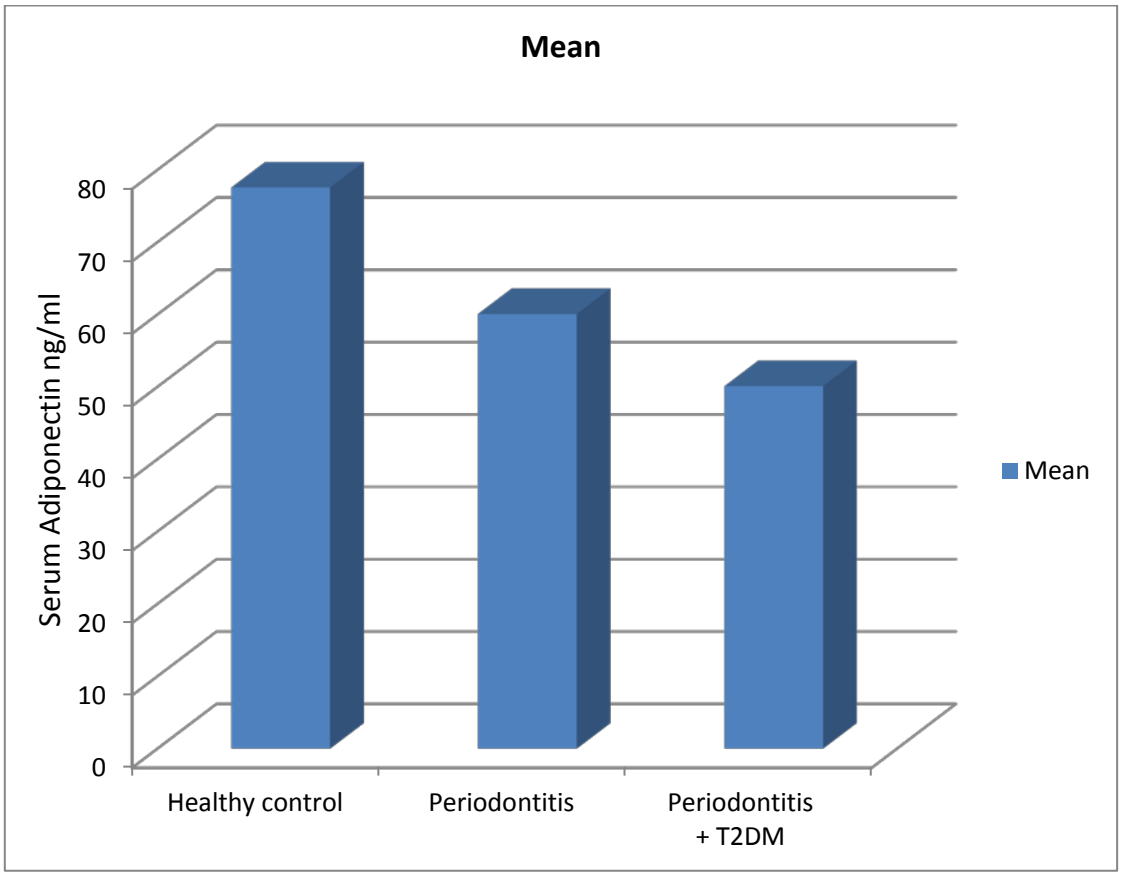


Figure 3.3: The differences in mean serum levels of adiponectin among study groups.

3.4 Serum ratio of leptin\ adiponectin

Determination the ratio of leptin\ adiponectin in current study revealed, that there was significant differences between patients group and controls group. The mean serum level of leptin\ adiponectin ratio in periodontitis group (0.43±0.08) and periodontitis+T2DM group (0.64±0.03) are significantly higher ($P<0.05$) when compared to the ratio in healthy control (0.21±0.02), as clearly show in table (3.5) and figure (3.4). On the other hand, when comparing between the two groups of patients, the present results found that there are significant elevation in the mean serum levels of leptin\ adiponectin ratio in periodontitis+T2DM group than that in periodontitis group, ($P<0.05$).

The fat tissue, especially the visceral type, acts as an important endocrine organ secreting several bioactive substances, such as adipokines. Among the most important ones are the tumor necrosis factor-alpha, leptin, adiponectin and resistin, which may modulate the periodontal response (Martin *et al.,* 2008). Several studies have reported associations between the ratio of leptin/adiponectin and insulin resistance. An increased leptin/adiponectin ratio has been shown related to insulin resistance in obesity,T2DM or metabolic syndrome. Since leptin and adiponectin are also involved in the inflammatory process, these biomarkers might play some roles in prediction of chronic inflammations (Zaletel *et al.,* 2010).

The results of the present study is similar to study performed by Jing Ling *et al* ., (2014), who found that the higher serum leptin/adiponectin ratio in patients of periodontitis with T2DM and periodontitis patients without T2DM than the healthy control people, and they concluded that periodontitis could influence the level of adipokines in serum and change the leptin/adiponectin ratio, and the effect would be enhanced combining with T2DM.

Saito and Shimazaki (2007) suggested that obesity, type 2 diabetes, and chronic periodontitis are closely associated, exhibiting a triangular relationship and the adipokines might play an important role in this association.

Tajtakova *et al.*, 2010, indicated that ratio of leptin/adiponectin could be a useful index of insulin resistance and ratio of leptin/adiponectin correlates with glucose infusion rate more closely than leptin and adiponectin alone.

Table 3.5: The differences in mean serum leptin\ adiponectin ratio among study groups.

Serum Adiponectin	Healthy control N=25	Periodontitis N=30	Periodontitis+T2DM N=30	p-value
Range	(0.01-0.73)	(0.01-3.98)	(0.03-2.2)	**0<0.05**
Median	0.15	0.31	0.32	
Mean	0.21	0.43	0.64	
SD	0.02	0.08	0.03	
Control Vs Periodontitis<0.05* Control Vs Periodontitis+T2DM <0.05* Periodontitis Vs Periodontitis+T2DM <0.05*				

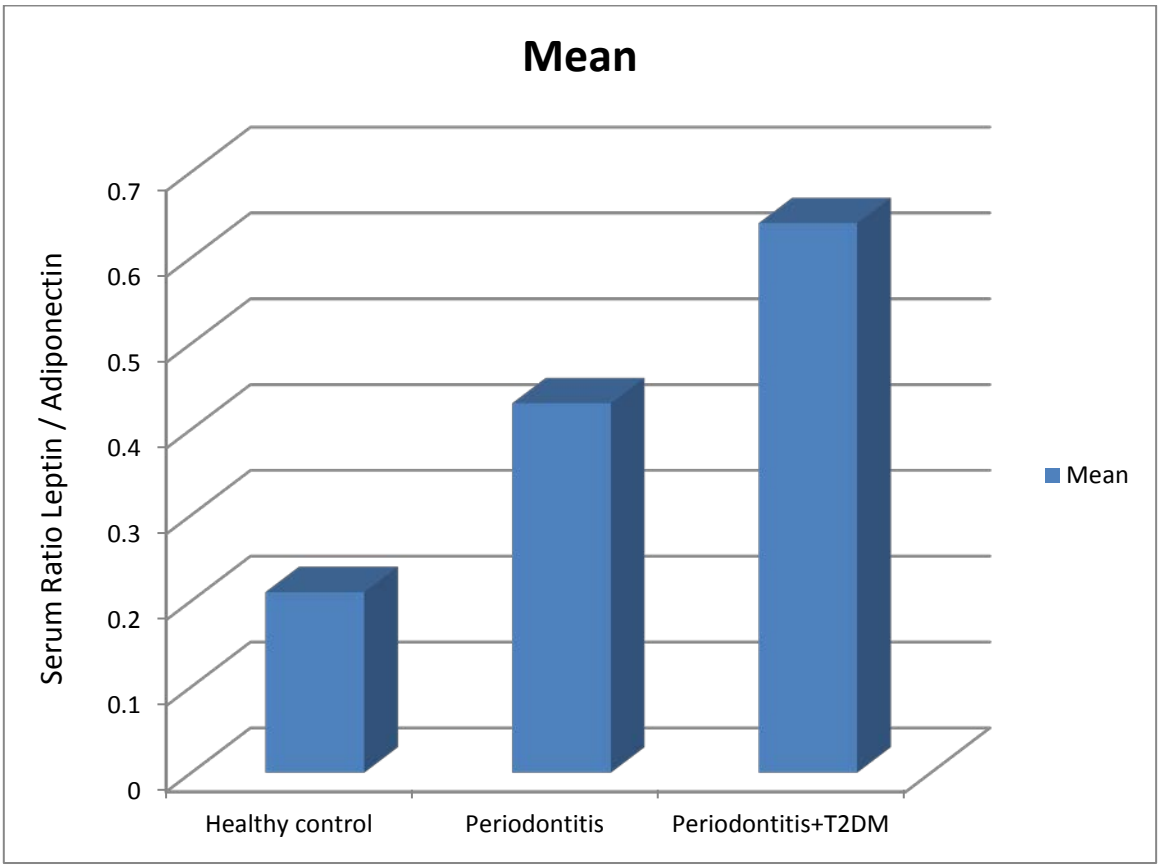


Figure 3.4: The differences in mean serum leptin\ adiponectin ratio among study groups.

3.5 Correlation between leptin and adiponectin

The results of correlation between leptin and adiponectin are clearly shown in figures (3.5) and (3.6). An anticipated, serum leptin level was showed significant negative correlation with serum adiponectin in both groups of patients; in periodontitis group patients was (r=-0.325, p=0.011) and in periodontitis+T2DM group of patients was (r=-0.434, p=0.017).

The adipose tissue secreated a varity of adiokines like adiponectin and leptin which are involved in endocrine processes regulating glugose and fatty metabolism , inflammatory response , immunity, cardiovascular function.(Teta *et al* ., 2008)**.** For many years the correlation between serum adiponectin and leptin in patients with periodontitis and T2DM remains unknown. The present result concordant with another study done by Jing Ling *et al* in (2014), who showed that the level of serum leptin was increased in both groups of patients when compared to healthy group, while the level of serum adiponectin was decreased in patients groups as compared to the healthy group.

Adel and Hala (2012), indicated that the serum leptin levels were significantly higher and the serum adiponectin levels were significantly lower in the T2DM group compared to the healthy control group which lead to highly significant negative correlation between leptin and adiponectin levels.

Correspondingly, Chan-Hee *et al* in (2013), observed that there was inverse correlation between serum adiponectin and leptin in Korean patients with T2DM. Conversely, Putz and colleagues showed that there was no association was found between serum adiponectin and serum leptin levels in T2DM patients (Putz *et al*., 2004).

Finally, the imbalance between adiponectin (anti-inflammatory) and leptin (pro-inflammatory) in periodontitis with T2DM diseases determine the degree of inflammation which can lead to major clinical effects.

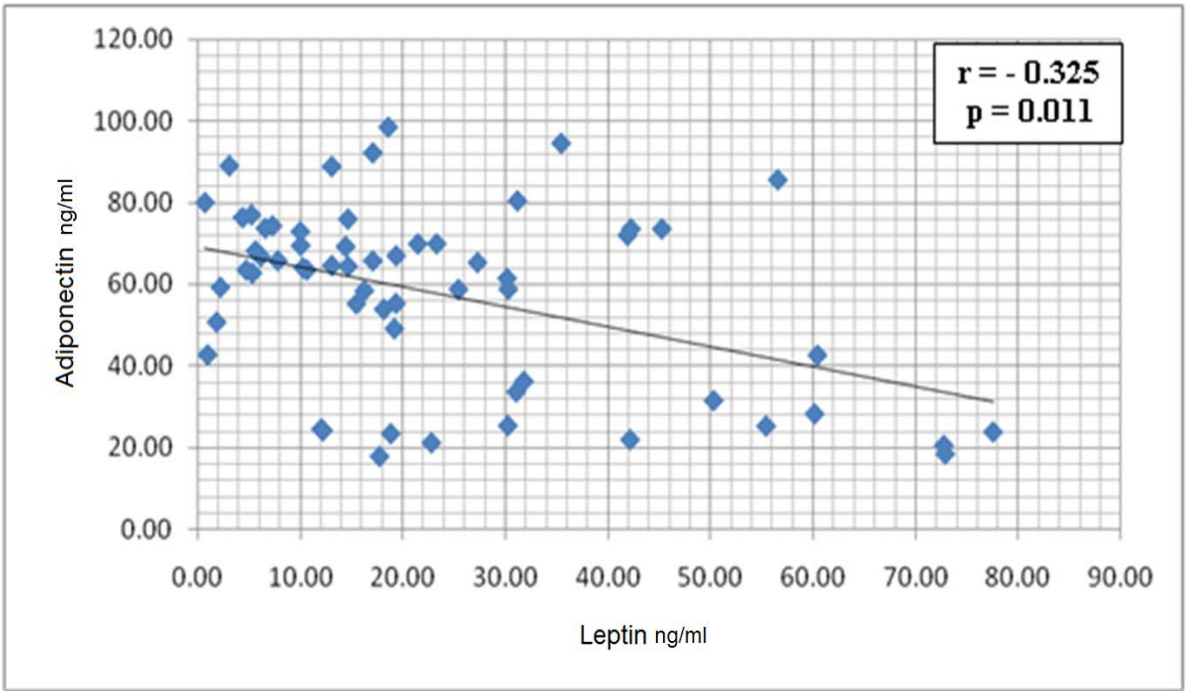


Figure 3.5: Correlation between leptin and adiponectin in periodontitis patients group

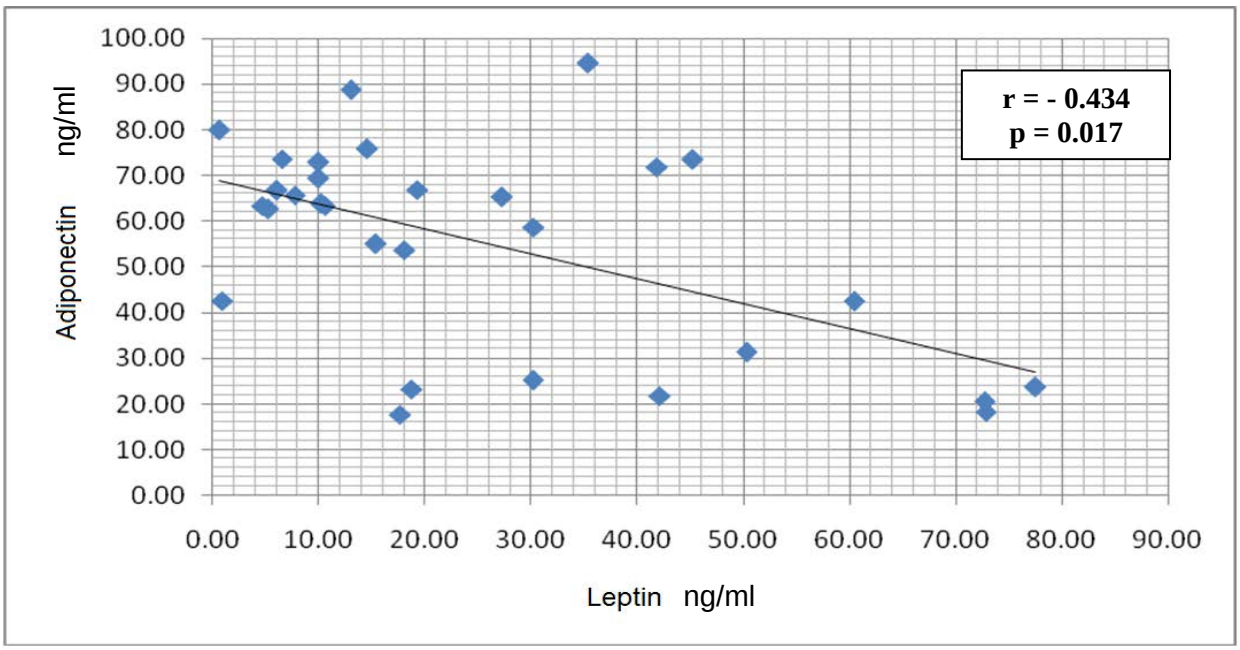


Figure 3.6: Correlation between leptin and adiponectin in periodontitis+T2DM patient group

3.6 Correlation between (leptin and adiponectin) and periodontal parameters in patients.

Spearman correlation coefficient test between serum (leptin, adiponectin, and ratio of leptin/adiponectin) and clinical periodontal parameters (PI, GI, PPD, CAL and BOP) were shown in tables (3.6, 3.7, 3.8). The current study did not observe any significant correlation between serum level of leptin, adiponectin, and ratio of leptin/adiponectin and clinical periodontal parameters ($p>0.05$), however; there is weak positive non significant correlation between serum level of leptin and PPD ($r=0.281$; $p= 0,083$), table (3.6).

However; results of present study are inconsistent with other results reported by (Karam, 2013; Jing Ling *et al*., 2014), they stated that there was asignificant positive correlation between serum leptin levels and some of periodontal parameters . Meanwhile Shimada *et al.*, (2010) found that serum leptin level was associated with mean probing depth, mean clinical attachment level and mean alveolar bone loss in the study population. So they concluded that this may be due to differences in disease stage between patient or that leptin levels is correlated to the degree of inflammation present and with no association to the degree of periodontal destruction represented by CAL this seems to be the possible logical explanation.

On the other hand, Jing Ling and associates found that the concentration of serum adiponectin was significantly negative correlated with periodontal parameters and the ratio of leptin/ adiponectin showed strong positive correlation with clinical periodontal parameters (Jing Ling *et al.*,2014).

The discrepancies observed between various studies could be caused, in part, to the differences in the sample size of each study, differences in types of samples used for each study and differences in sampling methods.

Table 3.6: Correlation between leptin level and clinical periodontal parameters.

Leptin	r-value	P-value
PI	0.160	0.397
GI	-0.147	0.438
PPD	0.281	0.083
CAL	-0.083	0.663
BOP	-0.081	0.671

Table 3.7: Correlation between adiopnectin and and clinical periodontal parameters.

Adiponectin	r-value	P-value
PI	-0.133	0.482
GI	-0.224	0.093
PPD	0.008	0.965
CAL	0.090	0.636
BOP	-0.032	0.868

Table 3.8: Correlation between Leptin/Adiponectin ratio and clinical periodontal parameters.

Leptin/Adiponectin	r-value	P-value
PI	0.137	0.469
GI	-0.083	0.663
PPD	0.057	0.764
CAL	-0.166	0.381
BOP	-0.127	0.503

Chapter Four

Conclusions and Recommendations

4.1 Conclusions

1. In the light of the present study, periodontitis could influence the serum levels of leptin and adiponectin, however; the effect would be enhanced combining with T2DM.
2. The significant elevation in serum level of leptin and reduction in adiponectin among patient groups as compared to a healthy control group may play a crucial role in pathogenesis of periodontitis.
3. The ratio of serum leptin / adiponectin could be applied as a new laboratory marker for insulin resistance in T2DM patients with periodontitis, moreover, the relative leptin / adiponectin ratio appears to be indicative of periodontitis occurrence.
4. Imbalance between pro and anti-inflammatory adipokines could be involved in the initiation and progression of periodontitis and is indicative of a stronger systemic proinflammatory state of disease.

4.2 Recommendations

1. Further studies are required to clarify the role of leptin and adiponectin in other samples, e.g., saliva and gingival crevicular fluid.
2. Studies are needed with a larger sample size to investigate the levels of serum leptin and adiponectin in periodontitis patients with other systemic diseases.
3. More studies are needed to evaluate the role of other adipokines such as resistin and vastin in the pathogenesis of periodontitis.
4. Application of molecular technique presents a new impetus in the study of inflammatory mediators and adipokines profile in periodontal diseases with and without T2DM.
5. Other studies are recommended to investigate the difference between adiponectin and leptin levels in periodontitis patients with other systemic diseases according to gender.
6. Since periodontitis is a local inflammatory disease, which also has some systemic effects, so study the correlation between adiponectin and leptin levels with bacterial accumulation in the dental plaque is recommended.

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Appendices

Appendix (I)

Case sheet for patients

Case No:

Name:

Age:

Gender:

Weight:

Length:

BMI:

Family history of disease:

Presence of DM:

Period of infection:

Taking drug:

F.B.S:

Clinical Parameter

* Plaqe index:

* Gingival index:

* Probing pocket depth:

* Clinical attachment level:

* Bleeding on probing:

* Number of teeth:

Appendix (II)

أني الموقع ادناة السيد اتعهد بموافقتي

علئ المشاركة في البحث (دراسة مقارنة لمستويات الاديبونكتين و اللبتين لمرضى التهاب اللثة المصابين وغير المصابين بداء السكري النوع الثاني) وذلك بقبولي علئ سحب عينة الدم مني من قبل طالبة الماجستير (فيان صباح علي) في المستشفئ التعليمي / كلية طب الاسنان/ جامعة بغداد - بعد التاكد بعدم وجود اي تاثيرات جانبية عند سحب الدم

أسم المريض:

التوقيع:

ألتاريخ:

Appendix (III)

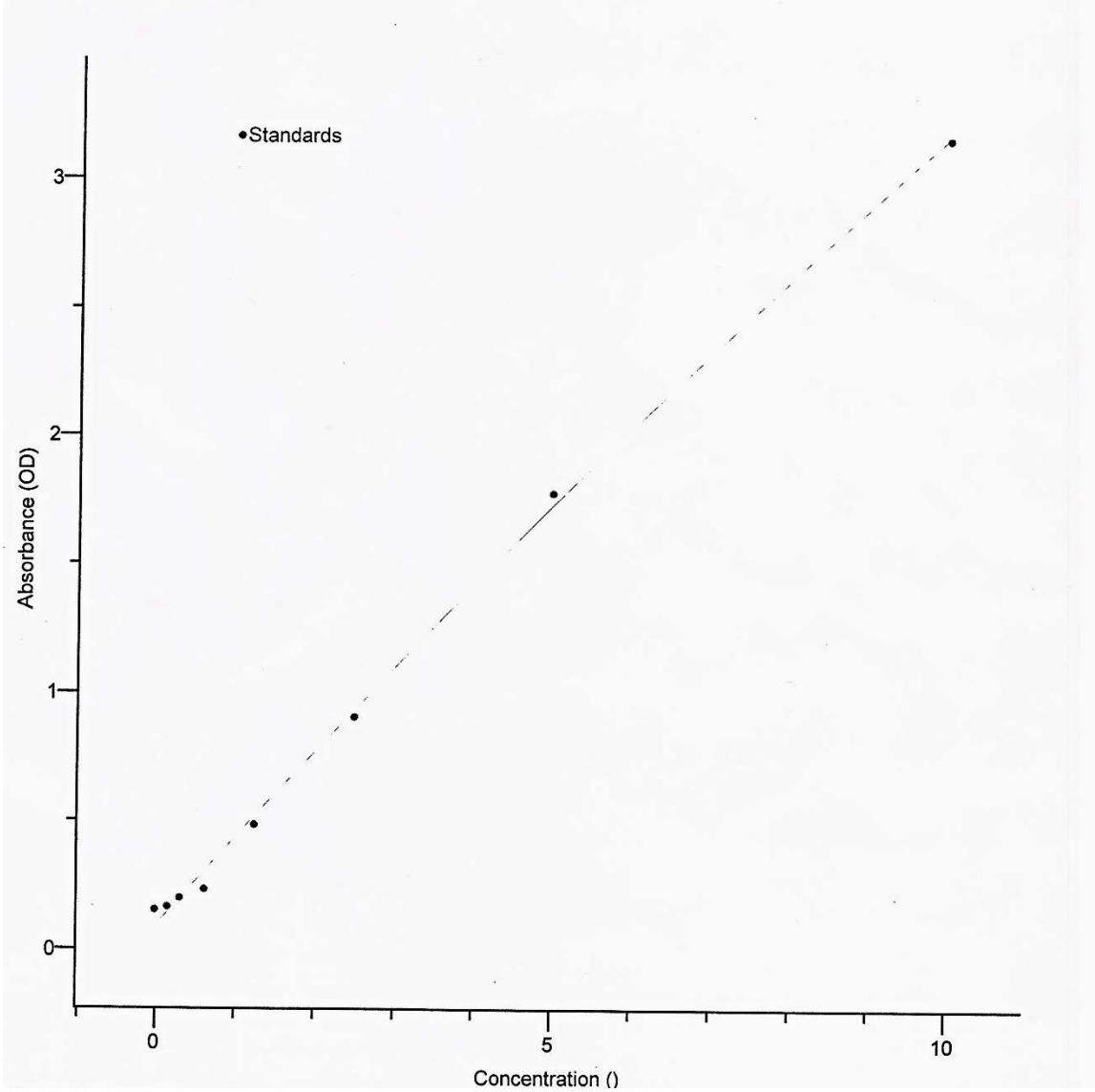
The International Classification of adult underweight, overweight and obesity according to BMI

Classification		BMI(kg/m²)	
Principal Classification	**Additional Classification**	**Principal cut-off points**	**Additional cut-off points**
Underweight		**<18.50**	**<18.50**
	Severe thinness	<16.00	<16.00
	Moderate thinness	16.00 - 16.99	16.00 - 16.99
	Mild thinness	17.00 - 18.49	17.00 - 18.49
Normal range		**18.50 - 24.99**	**18.50 - 22.99** **23.00 - 24.99**
Overweight		**≥25.00**	**≥25.00**
	Pre-obese	25.00 - 29.99	25.00 - 27.49 27.50 - 29.99
Obese		**≥30.00**	**≥30.00**
	Obese class I	30.00 - 34.99	30.00 - 32.49 32.50 - 34.99
	Obese class II	35.00 - 39.99	35.00 - 37.49 37.50 - 39.99
Severe obesity	Obese class III	**≥40.00**	**≥40.00**
	Morbid obesity	40.00 - 49.99	40.00 - 49.99
	Super obesity	≥50	≥50

Adapted from WHO, 1995, WHO, 2000 and WHO 2004

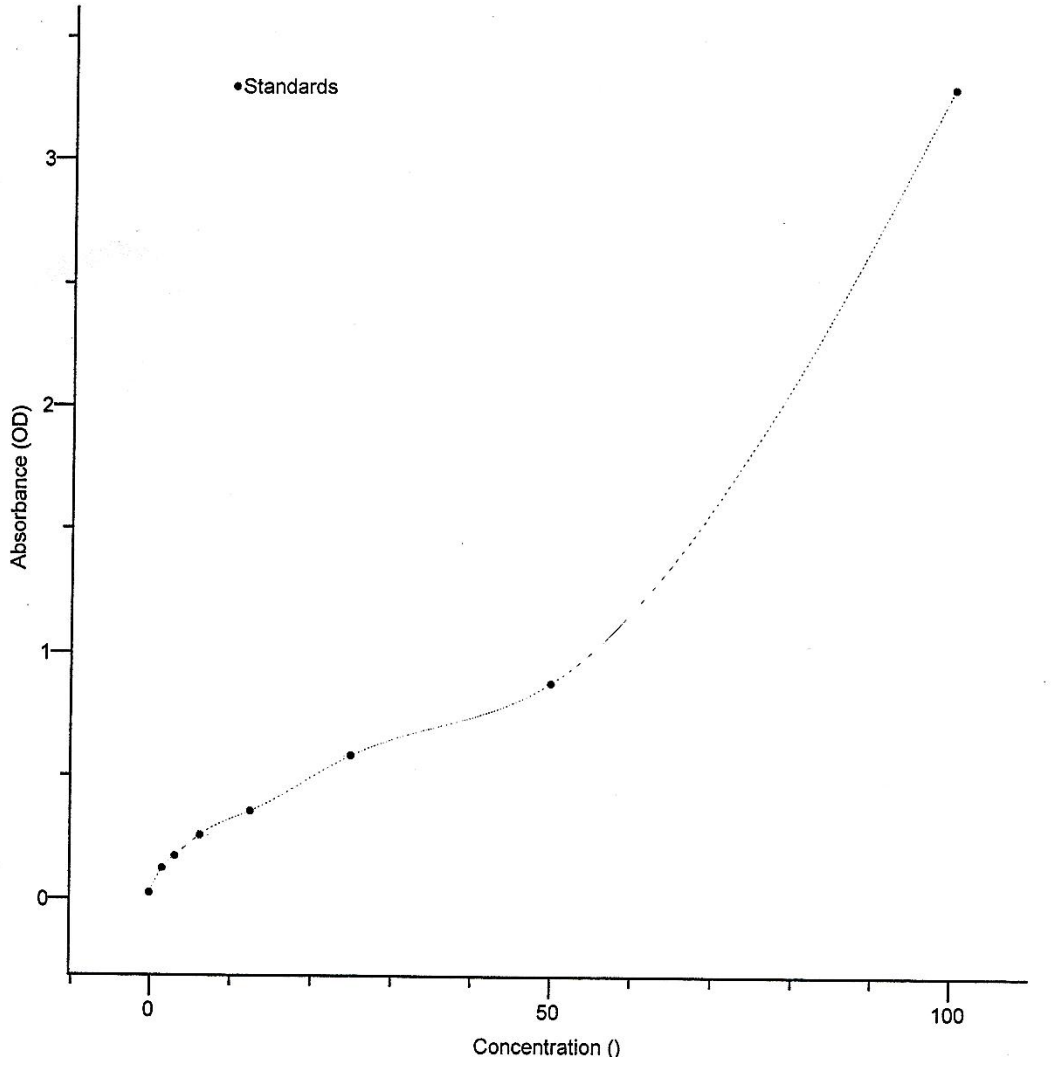
Appendix (IV)

Serum Leptin Elisa results (stander curve)



Appendix (V)

Serum Adiponectin Elisa results (stander curve)



جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم

دراسة مقارنة لمستويات الاديبونكتين و اللبتين لمرضى التهاب اللثة المصابين وغير المصابين بداء السكري النوع 2

رسالة

مقدمة الى كلية العلوم/ جامعة النهرين

كجزء من متطلبات نيل درجة الماجستير علوم/ تقانة احيائية

من قبل

قيان صباح علي العزاوي

بكالوريوس علوم التقانات الكيميائية الاحيائية / العلوم التطبيقية / الجامعة التكنلوجية

(2007-2006)

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أستاذ مساعد

رمضان 1435

حزيران 2014

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

إِنَّا فَتَحْنَا لَكَ فَتْحًا مُبِينًا (1) لِيَغْفِرَ لَكَ اللَّهُ مَا تَقَدَّمَ مِنْ ذَنْبِكَ وَمَا تَأَخَّرَ وَيُتِمَّ نِعْمَتَهُ عَلَيْكَ وَيَهْدِيَكَ صِرَاطًا مُسْتَقِيمًا (2) وَيَنْصُرَكَ اللَّهُ نَصْرًا عَزِيزًا (3)

صدق الله العظيم

سورة الفتح (1-3)

الاهداء

***الئ من الجنة تحت أقدامها.....الئ من تعجز الاقلام عن وصفها.....الئ من ساندتني بايمانها وصبرها حتئ لحظة رحيلها.....الئ اعز أنسانة في قلبي وحياتي أهدي ثمرة جهدي الئ روحك الطاهرة.**

أمي الغالية

***الئ الذي امطر جبينه رحقيأ أستمد منه قوتي..... وانار دربي بالامل الئ مثلي الاعلئ**

أبي الغالي

***الئ أساتذتي الفاضلين د. عبدالواحد شمخي و د. بتول حسن هاشم يشدني واجب الوفاء والاخلاص..... بالشكر والتقدير وألامتنان لدعمهم وتشجيعهم المتواصل**

***ألئ النفوس التي رسمت بالندئ الق الربيع.....الئ كل قلبأ خفق حبأ ووفأ لي**

اخواتي واخوتي

***ألئ كل الايادي التي ساندتني ..... لكي أصل الئ ماأنا عليه**

أهدي ثمرة جهدي

فيان

الخلاصة

تنشا امراض ما حول اللثة (النساغ) عن طريق الصحيفة الجرثومية ، التي تتراكم في منطقة التلمية والتي تحث الاستجابة الالتهابية. حيث وجدت الدراسات مؤخرا أن امراض ماحول اللثة (النساغ) قد تكون ذات صلة إلى العديد من الأمراض الجهازية وخاصة داء السكري.

في السنوات الأخيرة، زاد الاهتمام في دور الأنسجة الدهنية المشتقة من المواد التي تدعى الآديبوكين ودورها في الأمراض الالتهابية للإنسان بما في ذلك التهابات ماحول اللثة (النساغ). حيث تهدف هذه الدراسة لتقييم مستوى كل من هرمون الليبتين والاديبونيكتين من مصل الدم للمصابين بالتهاب النساغ مع اوبدون داء السكري النوع الثاني وتحديد العلاقة بين مستوى المعلمات البايوكيميائية (اللبتين والاديبونيكتين) مع المؤشرات السريرية لمرض التهاب النساغ والتحقق من العلاقة بين اللبتين والاديبونيكتين.

اجريت الدراسة علئ (60 مريض) مصابين بالتهاب النساغ (30شخص مصاب فقط بالتهاب النساغ و 30شخص مصاب بالتهاب النساغ مع داء السكري النوع الثاني) تراوحت اعمارهم مابين (32-64 سنة) ومقارنتهم مع (25 شخص سليم) كمجموعة سيطرة وكانت اعمارهم واجناسهم متقاربة مع المرضى . درست المؤشرات السريرية لمرض التهاب النساغ اذ استخدمت بعض العوامل مثل (موشر التهاب اللثة , مؤشر الصفيحة الجرثومية, مؤشر النزيف اثناء الفحص بالمسبار, عمق جيوب اللثة وفقدان الانسجة الرابطة سريرياً) تم جمع عينات دم من جميع المرضى والاصحاء ومن ثم فصل المصل من الدم لتقدير مستويات هرمون الليبتين والاديبونيكتين باستخدام تقنية الانزيم الممتز المناعية (Enzyme Linked Immuno Sorbent Assay).

كشفت الدراسة وجود ارتفاع معنوي ($p < 0.01$). في متوسط مستوى كل من هرمون الليبتين في مجموعتي التهاب النساغ مع أو بدون داء السكري النوع الثاني (32.16 ±**7.78** نانوغرام/مليليتر و 5.52±25.89 نانوغرام /مليليتر علئ التوالي) مقارنة مع الاشخاص الاصحاء (16.66 ± 3.93 نانوغرام/مليليتر)، علاوة على ذلك، أظهرت المقارنة بين مجموعتي المرضى أن متوسط مستوى هرمون الليبتين كان اعلئ في مرضئ التهاب النساغ الذين مصابين بداء السكري النوع الثاني ولكن إحصائيا غير معنوي ($p > 0.05$).

من ناحية أخرى، هناك انخفاض كبير ($p < 0.01$) في متوسط مستوى هرمون الاديبونيكتين في كل من مجموعتي التهاب النساغ مع أو بدون داء السكري النوع الثاني (50.10 ± 7.64 نانوغرام/مليليتر و 60.08 ±9.61 نانوغرام /مليليتلر ، على التوالي) عند مقارنتهم مع الاشخاص الاصحاء (77.57 ± 10.80 نانوغرام/مليليتر)، بالإضافة إلى ذلك، هناك انخفاض معنوي طفيف في متوسط مستوى هرمون الاديبونيكتين في المرضئ المصابين التهاب النساغ الذين مصابين بداء السكري النوع الثاني بالمقارنة مع أولئك المرضى المصابين بالتهاب النساغ فقط, ($p > 0.05$).

كما اظهرت النتائج ان نسبة اللبتين \ الاديبونيكتين أعلى بكثير في مجموعة التهاب النساغ مع أو بدون داء السكري النوع الثاني (0.64 ± 0.03 و0.43 ± 0.08) عند مقارنة مع النسبة في الاشخاص الاصحاء (0.21 ± 0.02)، (P <0.01). وقد لوحظ وجود علاقة سلبية مثيرة للاهتمام بين اللبتين والاديبونيكتين في المرضى الذين يعانون التهاب النساغ فقط (r = -0.325، P= 0.011) ومرضئ التهاب النساغ الذين مصابين بداء السكري النوع الثاني (r= -0.434،p=0.017).

أما بشأن العلاقة بين قيم (اللبتين والاديبونيكتين) والمؤشرات السريرية لمرض التهاب النساغ ، فإن الدراسة الحالية لم تظهر أي ارتباط كبير بين مستوى اللبتين، اديبونيكتين، ونسبة اللبتين / الاديبونيكتين مع المؤشرات السريرية لمرض التهاب النساغ (p> 0.05).

الاستنتاج من الدراسة الحالية هي أن مستويات كل من هرمون الليبتين والاديبونيكتين تلعب دورا مهما في التسبب بالتهاب النساغ مع أو بدون داء السكري النوع الثاني، وتظهر نسبة اللبتين / الاديبونيكتين النسبية لتكون مؤشرا دالأ على حدوث المرض. علاوة على ذلك ان عدم التوازن بين مستويات المدورات الخلوية الحاثة والمضادة للالتهابات قد تشارك في بدء وتطور مرض النساغ وكذلك يدل على وجود حالة التهابية جهازية قوية في هذا المرض.