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علم الحيوان

Leptin Status and some Biochemical Parameters among type 2 Diabetic Females in the Gaza Governorate, Gaza Strip.

حالة اللبتين وبعض العوامل البيوكيميائية لدى مرضى السكرى النوع الثاني من الاناث في محافظة غزة ، قطاع غزة

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بسم الله الرحمن الرحيم

Dedication

To my parents who have always supporting me

To my husband Yousef who helped me to accomplish this thesis.

To my beloved sons, Mohammed, Abed El Rahman and Omar

To my brothers and sisters

To my university IUG which is continuously improving the research

To all of them I dedicate this work

Hanan J Altawil

Declaration

I certify that this submission is my own research and that, to the best of my knowledge and belief, it contains material neither previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of the university of other institute, except where due a acknowledgment has been made in the text.

Signature	Name	Date
Hanan	Hanan J Altawil	July, 2009

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Leptin Status and some Biochemical Parameters among type 2 Diabetic Females in the Gaza Governorate, Gaza Strip.

Abstract

Background: Diabetes mellitus is a multifactorial disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Its prevalent rate in Gaza Strip is alarming. Although the role of leptin hormone in obesity is well established, its status in diabetes is still unclear and controversial. Understanding such role may help in future control and therapy of diabetes.

Objective: To assess leptin status and some biochemical parameters among type 2 diabetic females in Gaza Governorate, Gaza Strip.

Materials and Methods: In this cross sectional study, data were obtained from questionnaire interview, and biochemical analysis of blood and urine of 81 type 2 diabetic patients and 74 healthy individuals.

Results: The average age of the controls was 48.0±5.8 years whereas that of diabetic patients was 52.2±6.1 years. There was a significant decrease in the mean of blood glucose level with increasing the patient educational level. (F_{ANOVA}=2.82, P=0.027). Diabetes was found to be associated with family history (χ^2=3.2, P=0.05). Also, there was a high significant association between diabetes and diet (χ^2=95.93, P=0.000). The main self-reported complications among patients were retinopathy, neuropathy and cardiovascular diseases. In general, the longer the duration of diabetes, the higher the percentage of self reported complications among patients ($p<0.05$). Body mass index (BMI) was positively associated with diabetes ((χ^2=6.11, P=0.047). Serum glucose was significantly higher in diabetics compared to controls (mean=197.6$\pm$81.6mg/dl vs 89.9$\pm$14.2mg/dl, % difference=119.8, p=0.000). Serum urea and creatinine were not changed (mean=24.0±7.9mg/dl vs 24.9±7.8mg/dl and 0.73±0.23mg/dl vs 0.70±0.16mg/dl, % differences=3.6 and 4.3, p=0.492 and 0.257, respectively). Cholesterol, triglycerides(TAG) and low density lipoprotein(LDL-C) cholesterol

were significantly higher in diabetics (204.3±52.6mg/dl, 187.7±103.2mg/dl and 105.9±35.2mg/dl) than controls (180.5±36.1mg/dl, 118.3±72.7mg/dl and 91.9±31.5mg/dl) with % differences of 13.2, 58.7 and 15.2%, p=0.002, 0.000 and 0.011, respectively. In contrast, high density lipoprotein cholesterol (HDL-C) was significantly lower in diabetics (43.1±13.1mg/dl vs. 47.9±16.6mg/dl, % difference=10.0 and p=0.045). Serum leptin levels were significantly lower in diabetic patients compared to controls (12.3±8.7ng/ml vs. 16.8±14.4ng/ml, % difference=26.8 and p=0.018). Leptin was negatively correlated with blood glucose and triglyceride levels (r= -0.170, p = 0.030 and r= -0.174, p = 0.032) whereas it correlates positively with HDL-C level (r= -0.200, p = 0.013). Individuals who were not restricted to diet had higher leptin levels than those who did (t=1.66, p=0.07). The larger the BMI, the higher the level of leptin (F= 4.45, p = 0.013). Diabetic patients showed higher albumin levels in their urine compared to controls (41.1±62.6µg/dl vs. 24.7±23.5µg/dl,5, %difference=66.4 and p=0.045. serum creatinine level was normal (75.1±40.3mg/dl vs. 80.0±49.8mg/dl, %difference=6.1 and p=0.547). However, when albumin/creatinine ratio was calculated and averaged, significant increase was detected (0.72±1.11µg/mg vs. 0.37±0.41µg/mg, % difference=94.6 and p=0.017). Microalbuminuria among controls and patients were 7 (9.5%) and 20 (24.7%) with χ^2=3.59, P=0.045.

Conclusions: Diabetes is associated with family history, diet and obesity. Lower leptin levels were found in diabetics. Leptin was negatively correlated with blood glucose and triglyceride and positively with BMI and HDL-C. Diabetic patients showed higher albumin levels in their urine. Serum creatinine level was normal in blood and urine. Albumin/creatinine ratio increased in patients, as well as. Microalbuminuria.

Keywords: Biochemical parameters, Gaza Strip, Leptin, Type 2 diabetes.

ملخص الدراسة

اللبتين وبعض المعايير البيوكيميائية لدى مرضى السكرى النوع الثاني في الإناث في محافظة غزة ، قطاع غزة

مقدمة: السكري هو مرض متعدد الجوانب يتميز بفرط سكر الدم الناجم عن خلل في إفراز الإنسولين ، اوعمل الانسولين أو كليهما وانتشار المرض.بالمعدل السائد في قطاع غزة ينذر بالخطر. بالرغم من أن دور هرمون الليبتين في السمنه واضح، الا انه في مرض السكرى لايزال غامض ومثير للجدل، وفهم هذا الدور قد يساعد في المستقبل في ضبط وعلاج مرضى السكري.

الهدف: تهدف الدراسة الى معرفة حالة اللبتين وبعض المعايير البيوكيميائية لدى مرضى السكرى من النوع الثاني من الإناث في محافظة غزة.

الطرق والادوات: منهج الدراسة هو منهج مقطعى اجرى على مرضى السكرى من النوع الثاني من الاناث بمحافظة غزة، والبيانات المستخدمة فى الدراسة تم الحصول عليها من خلال المقابله المباشره مع المرضى، والتحاليل الكيميائية للدم والبول لعينة الدراسة، والتى تشمل 81 من مرضى السكر من النوع الثانى و 74 من الاصحاء حيث كان متوسط العمر للعينة الضابطه هو 48.0±5.8 ومتوسط العمر لمرضى السكر هو 52.2±6.1 سنه.

النتائج: اوضحت النتائج ان هناك انخفاض تدريجي في متوسط مستوى السكر في الدم مع زيادة المستوى التعليمي للمريض وان مرض السكري يرتبط بالتاريخ العائلي والتغذية كما ان المضاعفات الرئيسية التى سجلت على لسان المرضى اوضحت وجود اعتلال الشبكية، واعتلال الأعصاب وأمراض القلب والأوعية الدموية. و.بصفة عامة زيادة مدة الاصابة بمرض السكر تزيد من ارتفاع نسبة المضاعفات بين المرضى، وان مؤشر كتلة الجسم يتناسب طرديا مع الاصابه بالسكرى كما ان مستوى الكرياتينين والبولينا لم تتغير فى الدم وان مستوى كل من الكولسترول والدهون الثلاثية والكولسترول منخفض الكثافه أعلى في مرضى السكرى عن العينه الضابطه، وبالمقابل فان الكولسترول عالى الكثافة كان أقل في مرضى السكرى عن العينه الضابطه، واوضحت الدراسة ان هرمون اللبتين مستواه أقل في مرضى السكرى عن العينه الضابطه ويوجد علاقه سلبية بين هرمون اللبتين ومستوى السكر في الدم وكذلك مع الدهون الثلاثية، في حين أنه يرتبط بإيجابية مع الكوليسترول عالى الكثافة، كما واوضحت نتائج الدراسة ان مستوى هرمون اللبتين عند الاشخاص الذين يتبعون الحمية الغذائية يكون اقل، منه عند من لا يتبعون الحميه الغذائية، وأن مستوى هرمون اللبتين ايضا يزداد بازدياد مؤشر كتلة الجسم،.واظهر مرضى السكري مستويات أعلى من البيله الالبيومينية الزهيده في البول مقارنة مع الاصحاء وان نسبة الكرياتينين لم تتغير في الدم والبول بين المرضى والاصحاء، وعند حساب متوسط نسبة الالبومين على الكراتينين فقد وجدت انها مرتفعة فى المرضى مقارنة بالاصحاء

الاستنتاجات : يرتبط مرض السكري بتاريخ الاسرة، والتغذية والبدانة. وأن نسبة اللبتين تكون منخفضة عند مرضى السكري، وهناك علاقه عكسية بين مستوى السكر في الدم والدهون الثلاثية، وعلاقه ايجابية بين اللبتين ومعامل كتلة الجسم والكولسترول عالى الكثافة، وأظهر مرضى السكرى مستويات أعلى من البيله الالبيومينية الزهيده في حين أن الكرياتينين لم يتغير في البول والدم، وكانت نسبة الالبومين الى الكرياتينين مرتفعه في المرضى.

الكلمات المفتاحية :المعايير البيوكيميائية، قطاع غزة، الليبتين، مرض السكري من النوع الثانى

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Chapter 1

Introduction

1.1 Overview

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Two major forms of diabetes were identified; type 1 and type 2. Lack of or severe reduction in insulin secretion due to autoimmune or viral destructions of β cells is responsible for type 1 diabetes, which accounts for 5-10% of diabetic patients. The more prevalent form, type 2 diabetes, accounts for more than 90% of cases (Olefsky, 2001). Type 2 diabetes usually begins as insulin resistance, a disorder in which the cells do not use insulin properly. As the need for insulin rises, the pancreas gradually loses its ability to produce it (Cohen, 2006).

Lack of insulin action and/or secretion in type 2 diabetes induces hepatic glucose output by inhibiting glycogen synthesis and stimulating glycogenolysis and gluconeogenesis then increased rates of hepatic glucose production result in the development of overt hyperglycemia, especially fasting hyperglycemia (Defronzo et al., 1992 and Michael et al., 2000). In such conditions, lipolysis in adipose tissue is promoted leading to elevated circulating levels of free fatty acids. Ketones are produced, and are found in large quantities in ketosis, the liver converts fat into fatty acids and ketone bodies which can be used by the body for energy (Botion and Green, 1999). In addition, excess fatty acids in serum of diabetics are converted into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins (Jaworski et al., 2007).

Overweight and obesity are the major risk factors for diabetes. Most of type 2 diabetic patients were found to be obese (El-Hazmi et al., 1997, Eberhart et al., 2004 and Yassin et al., 2009). Chronic obesity leads to increased insulin resistance that can develop into diabetes (Camastra, 1999). Other risk factors include poor diet, sedentary lifestyle, increased age; 21% of people over 60 years have diabetes and family history; diabetes tends to run in families (Fujita, 2009 and Pijl et al., 2009).

Prolonged elevation of blood glucose levels (chronic hyperglycemia) may develop diabetic complications including neuropathy; nerve damage especially in extremities (Dyck et al., 2002), nephropathy; kidney damage, kidney failure (Maeda and Shiigai, 2007), retinopathy; vision problems, blindness (The National Eye Institute, 2006) and cardiovascular disease; heart disease and increased risk of strokes (Marshall, 2006).

Leptin, a 16 kDa circulating hormone produced and released primarily by adipose tissue, exerts a regulatory control mechanism on food intake via inhibition of neuropeptide Y(NPY) and increases the basal metabolism rate with selectively promoting fat metabolism (Prieur et al., 2008). Leptin is cleared from plasma mainly by the kidney (Chabova et al., 1999). Leptin could be regulated by insulin (Susan, 2000). The link of Leptin with obesity and diabetes is unclear and controversial. However, secretion of leptin is impaired in diabetes. Many researchers found a decrease in leptin levels in Type2 diabetic patients (Liu et al., 1999, Tatti et al., 2001, Abdelgadir et al., 2002 and Sayeed et al., 2003). Others demonstrated increase in the hormone level in type 2 diabetic patients with diabetic nephropathy (Verrotti et al., 2001 and El Meligi et al., 2003).

Although diabetes mellitus is prevalent in the Gaza Strip (Ministry of Health. MOH, 2005), there is under-diagnosis and under-reporting of the disease. Biochemical data are only restricted to monitoring blood glucose level when the patient visits the clinic. The present study assessed leptin as well as other biochemical parameters in blood and urine of type 2 diabetic females in Gaza Governorate, Gaza Strip (G.S) Understanding the status of

leptin and other parameters could be useful in the management of the disease.

1.2 Significance

- In the G.S, few studies have been carried out on diabetes mellitus without speculation the role of leptin hormone in the disease (Altibi, 2007, Shubair, 2008 and Abu Hilal, 2009). In another study, leptin was investigated in nondiabetic obese patients (Al-Holi, 2006). This will be the first study to assess leptin status and relate it to blood glucose level and some other biochemical parameters among diabetic females in the Gaza City.
- Understanding the role of leptin in metabolic disorders may be helpful in control of DM.
- It is important to give a detailed picture on the contributing risk factors as well as on biochemical features of diabetes among type 2 diabetic females in the G.S.

1.3 Objective

The overall aim of the current study is to assess leptin status as well as some biochemical parameters in type 2 diabetic females in Gaza City.

Specific objectives

1. To identify the risk factors and self reported complications in type 2 diabetic females.

2. To assess leptin level in diabetics and controls and its relation to blood glucose level, diet, BMI and lipid profile.
3. To determine other biochemical parameters including urea, creatinine, cholesterol, triglycerides, high density lipoprotein cholesterol and low density lipoprotein cholesterol.
4. To evaluate microalbuminuria, creatinine and albumin/creatinine ratio in the patients compared to the controls

Chapter 2

Literature Review

2.1 Definition of diabetes mellitus

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.

2.2 Types of diabetes

2.2.1 Type 1 diabetes

It was previously called insulin-dependent diabetes mellitus or juvenile-onset diabetes. Type 1 diabetes develops when the body's immune system destroys pancreatic beta cells resulting in failure of insulin production. This form of diabetes usually strikes children and young adults, although disease onset can occur at any age. Type 1 diabetes accounts for 5-10% of all diagnosed cases of diabetes (Olefsky, 2001)

2.2.2 Type 2 diabetes

It was previously called non insulin-dependent diabetes or adult-onset diabetes. Type 2 diabetes results from insulin resistance, a condition in which the body fails to properly use insulin, combined with relative insulin deficiency (Robbins and Cotran, 2004). This form of diabetes accounts for about 90-95% of all diagnosed cases of diabetes. Type 2 diabetes is associated with older age, obesity, history of gestational diabetes, impaired glucose metabolism, physical inactivity, and race/ethnicity (Olefsky, 2001).

2.2.3 Gestational diabetes

It is diabetes that develops in the course of pregnancy in some women. It is common among obese women and women with a family history of diabetes.

Treatment is with diet in the first instance, but most patients require insulin cover during pregnancy to normalize maternal blood glucose levels to avoid complications in the infant. After pregnancy, 5-10% of women with gestational diabetes are found to have type 2 diabetes (Centers for Disease Control and Prevention, CDC, 2005).

2.2.4 Other types of diabetes

A. Genetic defects of the β-cell: these forms of diabetes are frequently characterized by onset of hyperglycemia at an early age (before age 25 years). They are referred to as maturity-onset diabetes of the young (MODY) and are characterized by impaired insulin secretion with minimal or no defects in insulin action. They are inherited in an autosomal dominant pattern e.g. MODY 1, 2, 3 (American Diabetes Association, 2005).

B. Genetic defects in insulin action: there are unusual causes of diabetes that result from genetically determined abnormalities of insulin action.

C. Diseases of the exocrine pancreas: any injures in the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma.

D. Endocrinopathies: hormones like growth hormone, cortisol, glucagons, and epinephrine antagonize insulin action.

E. Infections: certain viruses have been associated with β-cell destruction. Diabetes occurs in patients with congenital rubella, Coxsackie's B, cytomegalovirus, adenovirus, and mumps. (American Diabetes Association, 2005).

2.3 Prevalence of diabetes mellitus

Prevalence of diabetes mellitus is increasing worldwide, in line with lifestyle changes and population aging. In particular, the rising prevalence of diabetes is closely linked with that of obesity. World Health Organization estimates that

at least 177 million people worldwide suffer from diabetes and this figure is likely to be more than double by the year 2030 (WHO, 2003).

In the USA, there are an estimated 23.6 million people (7.8% of the population) with diabetes with 17.9 million being diagnosed (American diabetes Association, 2008), 90% of whom are type 2 (Inzucchi and Sherwin, 2007). With prevalence rates doubling between 1990 and 2005, CDC has characterized the increase as an epidemic (Gerberding, 2007).

Diabetes is the fourth leading cause of death in Europe, and it carries a 3-4 times higher risk of major cardiovascular complications and is now the commonest cause of heart attack and stroke and a major cause of peripheral vascular disease and peripheral neuropathy leading to a 20 fold higher risk of amputation. The cost of diabetes complications accounts for 5-10% of total healthcare spending in several countries including Belgium, France, Germany, Italy, the Netherlands, Spain, Sweden, and the UK. In today's Europe, the average prevalence rate of diabetes is 7.5%, and about 60 million people live with diabetes, of whom more than 50% are unaware of their condition leaving them exposed to the risk and cost of complications associated with poor control of the illness. The South-East Asian region has the highest number of people with diabetes mellitus with some 49 million, and its prevalence of 7.5% is the second highest, behind North America (7.8%), and ahead of the Eastern Mediterranean and Middle East regions (6.4%), (International Diabetes Federation, IDF, 2004).

In Palestine, the prevalence of diabetes mellitus was examined in a study conducted in 2000 in cooperation with Al-Quds University and Ministry of Health. The results indicated that the prevalence was about 9% (Ministry of Health, 2002). It is around the reported prevalence rate in Egypt and Tunisia (9%) and less than in Saudi Arabia (12%) and Oman (13%). However, in Palestine, there is under-diagnosis and under-reporting of the disease. This is due to lack of proper hospital and clinic recording system (MOH, 2005).

2.4 Mortality rate of diabetes mellitus in Palestine

Mortality rate of diabetes mellitus among Palestinians constituted 3.6% of total population deaths. A total of 372 persons died with mortality rate of 10.2 per 100,000 (males 9.5 per 100,000, females 10.9 per 100,000). The average annual mortality rate of diabetes mellitus increased to 12.4 per 100.000 populations in the last five years (Ministry of Health 2005).

2.5 Type 2 diabetes

2.5.1 Metabolism in type 2 diabetes

Circulating glucose is derived from 1) intestinal absorption during the fed state in which the rates of gastric emptying determine how quickly glucose appears in the circulation during the fed state, and from 2) hepatic processes including glycogenolysis (the breakdown of glycogen) and gluconeogenesis (the formation of glucose primarily from lactate and amino acids during the fasting state). Renal gluconeogenesis contributes substantially to the systemic glucose pool only during periods of extreme starvation. Although most tissues have the ability to hydrolyze glycogen, only the liver and kidneys contain glucose-6-phosphatase, the enzyme necessary for the release of glucose into the circulation.

The rate of glucose entering the circulation (glucose appearance) balanced by the rate of glucose removal from the circulation (glucose disappearance). The glucoregulatory hormones of the body are designed to maintain circulating glucose concentrations in a relatively narrow range. Glucoregulatory hormones include insulin, glucagon, amylin, glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic peptide (GIP), epinephrine, cortisol, and growth hormone. Of these, insulin and amylin are derived from the ß-cells, glucagon from the α-cells of the pancreas, and GLP-1 and GIP from the L-cells of the intestine.

In the bi-hormonal model of glucose homeostasis, insulin is the key regulatory hormone of glucose disappearance, and glucagon is a major regulator of glucose appearance. After reaching a post-meal peak, blood glucose slowly decreases during the next several hours, eventually returning to fasting levels. In the immediate post-feeding state, glucose removal into skeletal muscle and adipose tissue is driven mainly by insulin. At the same time, endogenous glucose production is suppressed by 1) the direct action of insulin on the liver, and 2) the paracrine effect or direct communication within the pancreas between the α- and ß-cells, which results in glucagon suppression.(Wallum et al., 1992).

Type 2 diabetes is a disorder characterized by lack of insulin action and/or secretion that induces hepatic glucose output by inhibiting glycogen synthesis and stimulating glycogenolysis and gluconeogenesis then increased rates of hepatic glucose production result in the development of overt hyperglycemia, especially fasting hyperglycemia (Defronzo et al., 1992 and Michael et al., 2000).

In such conditions, lipolysis in adipose tissue is promoted leading to elevated circulating levels of free fatty acids. Ketones are produced, and are found in large quantities in ketosis, the liver converts fat into fatty acids and ketone bodies which can be used by the body for energy (Botion and Green, 1999). In addition, excess fatty acids in serum of diabetics are converted into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins (Jaworski et al., 2007). Several studies showed that cholesterol, triglycerides and LDL-C are elevated in diabetic patients (Barrett-Connor et al., 1982,). In contrast, other studies documented that HDL-C was decreased (Altibi, 2007).

Reduced glucose disposal and fuel flux during hyperinsulinemic clamp in type 2 diabetic patients may affect mitochondrial ATP production, and ATP-dependent processes such as protein synthesis could be curtailed by reduced ATP availability. Association of insulin resistance with reduced ATP production occurs in aging (Short, 2005) in association with reduced muscle

mitochondrial protein synthesis. Insulin has effects not only at the translational level, but also at the transcription level of protein synthesis (Kimball SR 2004). The effect of insulin on protein synthesis is likely to be not only tissue specific but also protein specific (Boirie et al., 2001). In addition, kinetics of whole-body protein metabolism is elevated, and net balance is diminished in type 2 diabetes, independently of obesity (Gougeon et al., 2008).

2.5.2 Risk factors and symptoms of type 2 Diabetes

The most common risk factors for type 2 diabetes comprise obesity, poor diet, sedentary lifestyle, increased age; 21% of people over 60 years have diabetes and family history; diabetes tends to run in families (Fujita, 2009 and Pijl et al., 2009). Not everyone with type 2 diabetes has symptoms, particularly in the early stages of the disease. In fact, 5.7 million of the 23.6 million people with diabetes are unaware that they even have the disease. Of those, 90 to 95% are those with type 2 diabetes (CDC, 2008). However, type 2 diabetes symptoms may include one or more of the following:

- Excessive thirst
- Frequent urination
- Extreme hunger
- Unexplained weight loss
- Fatigue, or a feeling of being "run down" and tired
- Rapid breathing
- Blurred vision
- Dry, itchy skin
- Headache
- Tingling or burning pain in the feet, legs, hands, or other parts of the body
- High blood pressure
- Mood swings
- Irritability, depression

- Frequent or recurring infections, as urinary tract infections, yeast infections, and skin infections
- Slow healing of cuts and bruises

2.5.3 Complications of type 2 Diabetes

2.5.3.1 Heart conditions and stroke.

Cardiovascular disease is the number one killer of people with type 2 diabetes, people with diabetes developing certain problems with the heart and blood vessels. Some of these problems are Heart attack, stroke and blockage of blood vessels in the legs and feet, which can lead to foot ulcers, infections, and even loss of a toe, foot, or lower leg (Marshall, 2006).

Myocardial ischemia due to coronary atherosclerosis commonly occurs without symptoms in patients with diabetes. As a result, multivessel atherosclerosis often is present before ischemic symptoms occur and before treatment is instituted. A delayed recognition of various forms of coronary heart disease undoubtedly worsens the prognosis for survival for many diabetic patients. One reason for the poor prognosis in patients with both diabetes and ischemic heart disease seems to be an enhanced myocardial dysfunction leading to accelerate heart failure. Several factors probably underlie diabetic cardiomyopathy: severs coronary atherosclerosis, prolonged hypertension, chronic hyperglycemia, microvascular disease, glycosylation of myocardial proteins, and autonomic neuropathy. Improved glycemic control, better control of hypertension, and prevention of atherosclerosis may prevent or mitigate diabetic cardiomyopathy (Savage, 2005).

Several predisposing factors simultaneously affect the development of cardiovascular disease and diabetes mellitus. Among these concomitant factors are obesity, physical inactivity, heredity, sex, and advancing age. To some extent, these predisposing factors exacerbate the major risk factors: dyslipidemia, hypertension, and glucose in tolerance; and they may cause

cardiovascular disease and diabetes mellitus as well. To a large extent, both cardiovascular disease and diabetes mellitus must be prevented through control of predisposing risk factors. Modification of life habits is at the heart of the public health strategy for prevention of cardiovascular disease and diabetes mellitus. High priorities are the prevention (or treatment) of obesity and promotion of physical activity. Drug therapy nonetheless may be required to control the metabolic risk factors, particularly when they arise from genetic aberration and aging (Grundy, 1999)

2.5.3.2 Eye diseases

A. Retinopathy: involves changes in the retina, the light-sensitive layer at the back of the eye. These changes happen because of damage or growth problems in the small blood vessels of the retina. Usually, changes in the retinal blood vessels don't appear before a person has reached puberty and has had diabetes for several years. Retinopathy is more likely to become a problem in people with diabetes if they have high blood sugar levels and high blood pressure over a long period of time. One reason why diabetes needs to have regular yearly eye exams is because people with retinopathy may not have any problems seeing at first. But if the condition gets worse, they can become blind. A person with diabetes may be able to slow or reverse the damage caused by retinopathy by improving blood sugar control. If retinopathy becomes more advanced, laser treatment may be needed to help prevent vision loss (The National Eye Institute, 2006).

B. Glaucoma: People who have diabetes also have a greater chance of getting glaucoma. In this disease, pressure builds up inside the eye, which can decrease blood flow to the retina and optic nerve and damage them. At first, a person may not have trouble seeing. But if it's not treated, glaucoma can cause a person to lose vision. The risk increases as a person gets older and has had diabetes longer. People with glaucoma take medications to lower the pressure inside the eye and sometimes need surgery. The patient may

also recommend seeing an ophthalmologist or optometrist (The National Eye Institute, 2006).

2.5.3.3 Kidney disease

The kidneys have a range of functions and one of the most important is excreting waste products including urea from protein, uric acid from nucleic acids, creatinine from muscle creatine and many others. Creatinine and urea are used as markers of kidney functions (Debra Manzella, 2008). Therefore, changes in creatinine and urea levels are indicators of impairment of kidney function. In type 2 diabetes it is difficult to determine the onset of such changes and this may lead to controversial results (Varghese et al., 2001 and El Meligi et al., 2003). Once clinical kidney disease is evident, the rate of decline glomerular filtration rate (GFR) is highly variable, ranging from 2 to 20 ml min-1 yr-1 (American Diabetic Association, 2004). The reasons for these differences in the rate of disease progression are multifactorial, including both non-modifiable and modifiable factors (Ueda et al., 2003). Blood pressure control is known to be important in preventing adverse cardiovascular and renal outcomes in diabetic patients with hypertension (Rossing et al., 2004 and Bakris, 2004).

One of the most severe complications of diabetes is the development of diabetic nephropathy. The earliest detectable change in the course of diabetic nephropathy is a thickening in the glomerulus. At this stage, the kidney may start allowing more serum albumin (plasma protein) than normal in the urine (albuminuria), and this can be detected by sensitive medical tests for albumin. This stage is called microalbuminuria (urinary albumin 30-300 mg/24hr). It can appear 5 to 10 years before other symptoms develop. As diabetic nephropathy progresses, increasing numbers of glomeruli are destroyed by nodular glomerulosclerosis. Now the amounts of albumin being excreted in the urine increases, and may be detected by ordinary urinalysis techniques. The condition is called macroalbuminurea (urinary albumin >300 mg/24hr). At this stage, a kidney biopsy clearly shows diabetic nephropathy (Maeda and Shiigai, 2007). Further progress of the disease may lead to end

stage kidney disease. Most diabetic patients with end stage kidney disease have type 2 diabetes (American Diabetic Association, 2004).

2.5.3.4 Diabetic neuropathy

Diabetic neuropathy can affect nerves in many different parts of the body. The most common early symptoms of the condition are numbness, tingling, or sharp pains in the feet or lower legs. An estimated 50% of those with diabetes have some form of neuropathy, but not all with neuropathy have symptoms. The highest rates of neuropathy are among people who have had the disease for at least 25 years. Diabetic neuropathy also appears to be more common in people who have had problems controlling their blood glucose levels, in those with high levels of blood fat and blood pressure, overweight people, and people over the age of 40 (Dyck et al., 2002). If it's not treated, nerve damage can cause a number of problems. For example, because of the numbness, people with nerve damage might not realize that they have a cut, and it could become seriously infected before they discover it. Because nerve damage can happen anywhere in the body, problems can occur in almost any organ system, including the digestive tract, urinary system, eyes, and heart (Debra Manzella, 2006).

2.5.3.4 Other complications

They include foot problems and leg amputations, skin disorders, decreased cognitive abilities and dementia, sexual dysfunction, pregnancy complications, some types of cancer, yeast infections, urinary tract infections, gingivitis, thrush, tuberculosis and other infections. In addition, recent research has found an increased prevalence of asthma and Parkinson’s disease in people with type 2 diabetes (Debra Manzella, 2008).

2.6 Obesity

Obesity is a condition in which excess body fat has accumulated to an extent that health may be negatively affected. BMI is a measure of body fat based on a formula that calculates the ratio of body weight in Kg/height in meter square (National Heart, Lung and Blood Institute, 1998). Therefore, obesity is commonly defined as a BMI of 30 kg/m^2 or higher (WHO 2000) This definition distinguishes obesity from being pre-obese or overweight, which is classified as a BMI of 25 kg/m^2 but less than 30 kg/m^2 (Table 2.1)

Table 2.1 Weight classifications by body mass index (WHO, 2000).

Classification	**BMI**
Underweight	Less than 18.5
Normal weight	18.5-24.9
overweight	25.0–29.9
Obese I	30.0–34.9
Obese II	35.0–39.9
Extreme obesity	≥40

High body mass are major risk factors for a number of chronic diseases, including diabetes, cardiovascular diseases and cancer. Once considered a problem only in high income countries, overweight and obesity are now dramatically on the rise in low- and middle-income countries, particularly in urban settings (WHO, 2009). WHO's latest projections indicated that globally in 2005 approximately 1.6 billion adults (age 15+) were overweight, at least 400 million adults were obese. WHO further projects that by 2015, approximately 2.3 billion adults will be overweight and more than 700 million will be obese (WHO, 2006).

The fundamental cause of obesity and overweight is an energy imbalance between calories consumed on one hand, and calories expended on the other hand. Global increases in overweight and obesity are attributable to a number of factors including:

- a global shift in diet towards increased intake of energy-dense foods that are high in fat and sugars but low in vitamins, minerals and other micronutrients; and
- a trend towards decreased physical activity due to the increasingly sedentary nature of many forms of work, changing modes of transportation, and increasing urbanization.

High body mass lead to serious health consequences. Risk increases progressively as BMI increases. Raised body mass index is a major risk factor for chronic diseases including diabetes. About 55 percent of type 2 diabetics are obese (Eberhart et al., 2004). Chronic obesity leads to increased insulin resistance that can develop into diabetes, most likely because adipose tissue (especially that in the abdomen around internal organs) is a (recently identified) source of several chemical signals to other tissues (hormones and cytokines). Other research shows that type 2 diabetes causes obesity as an effect of the changes in metabolism and other deranged cell behavior attendant on insulin resistance (Camastra, 1999). Additional factors found to increase risk of type 2 diabetes include aging (Jack, 2004), high-fat diets (Lovejoy, 2002) and a less active lifestyle (Hu, 2003).

High body mass, as well as their related chronic diseases, are largely preventable. At the individual level, people can achieve energy balance and a healthy weight; limit energy intake from total fats and shift fat consumption away from saturated fats to unsaturated fats; increase consumption of fruit and vegetables, as well as legumes, whole grains and nuts; limit the intake of sugars; and increase physical activity - at least 30 minutes of regular, moderate-intensity activity on most days. More activity may be required for weight control (Debra Manzella 2008).

2.7 Leptin

2.7.1 Definition and site of secretion

Leptin -Greek *leptos* meaning thin- is a 16 kDa protein hormone that plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism (Figure 1). Leptin is one of the most important adipose derived hormones (Kiess, 1998). The *Ob (Lep)* gene (Ob for obese, Lep for leptin) is located on chromosome 7 in humans, the gene encodes adipose tissue mRNA with a highly conserved 167-amino acids. The amino-acid sequence of leptin is approximately 84% identical between human and mouse. A very small group of humans possess homozygous mutations for the leptin gene which leads to a constant desire for food, resulting in severe obesity. This condition can be successfully treated by the administration of recombinant human leptin (Zhang et al., 1994).

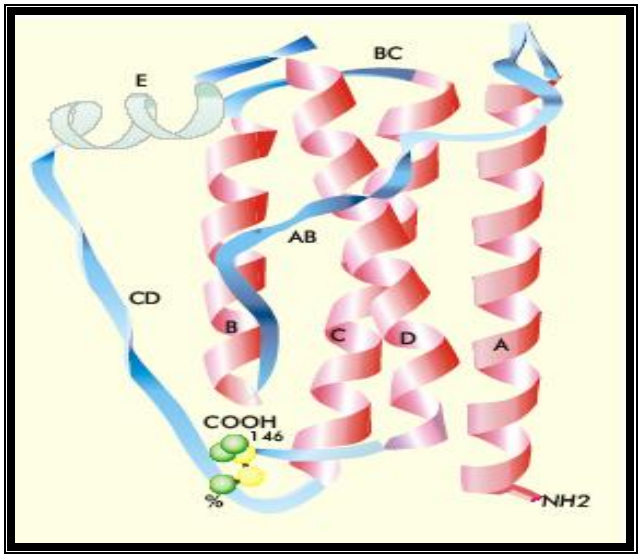

Figure 1: Structure of leptin(zhang, 1997)

It is reported that a number of a non adipocyte tissue have been shown to synthesize and secret low level of leptin including the gastric mucosa, mammary, epithelial cell, myocytes, placenta, testes, ovary, and hair follicles

(Bado, 1998) Leptin circulates in the plasma as free form or bound to leptin binding protein. These plasma binding proteins are likely to include a soluble form of leptin receptor .It has been suggested that the great majority of leptin circulates in the bound form in lean individual and in free form in obese subjects(Sinha, 1996).

The half-life of leptin is 9.4±3.0 min, and the leptin production rate was 3.6±1.2ng/100 g fat/min (Jianbo Zeng, et al, 1997). The human kidney plays a substantial role in leptin removal from plasma by taking up and degrading the peptide. Renal leptin uptake could account for ~80% of all leptin removal from plasma (Meyer, et al, 1997).

2.7.2 Mechanism of action of leptin

Leptin is produced by adipose tissue and interacts with six types of receptor (LepRa–LepRf) which in turn are encoded by a single gene, LEPR. (Wang et al., 1996). Ob-Rb is the only receptor isoform that contains active intracellular signaling domains required for activation of the JAK (Janus-Activated Kinase) - STAT3 (Signal Transducer and Activators of Transcription) and MAPK (Mitogen-activated protein kinases), this receptor is present in a number of hypothalamic nuclei (Malendowicz et al., 2006). Leptin binds to the ventromedial nucleus of the hypothalamus, known as the "appetite center" Leptin signals to the brain that the body has had enough to eat, or satiety (Nussey and Whiteheds, 2001).

It is not known if the leptin can cross the blood-brain barrier to access receptor neurons, because the blood-brain barrier is some what absent in the area of the median eminence, close to neuropeptide Y (NPY) neurons of the arcuate nucleus. Leptin might enter the brain at the choroid plexus, where there is intense expression of a form of leptin receptor molecule that could act as a transport mechanism. It is unknown whether leptin can cross the blood-brain barrier to access receptor expression of a form of leptin receptor molecule that could act as a transport mechanism (Margetic et al., 2002).

The arcuate nucleus (ARC) is rich in leptin receptor; leptin binds to its receptor where it activates the JAK/STAT3 pathway. STAT3 protein regulates NPY and pro-opimelanocortin (POMC) neurons (Figure 2). Leptin inhibits NPY and agouti-related peptide (ARGP) neurons in the ARC. Both NPY and ARGP are potent orexigenic agent .Leptin activates POMC neurons that produce melanocyte stimulating hormone (άMSH). άMSH acts at the melaocortin-4 (MC4) receptor and is co-expressed with the Cocaine pro-opimelanocortin and amphetamine regulated transcript (CART). POMC/CART neurons also project to the lateral hypothalamic nucleus (LH) and paraventricular vucleus (PVN) and there is reciprocal innervation from these nuclei to the ARC. Both άMSH and CART are potent anorexigenic agents. Increasing in NPY activity and reduction in POMC activity appears to increase feeding and fat deposition. Where as reduction in NPY activity and increasing in POMC activity decrease feeding and body mass (Janeckova, 2001 and Flier, 2004).

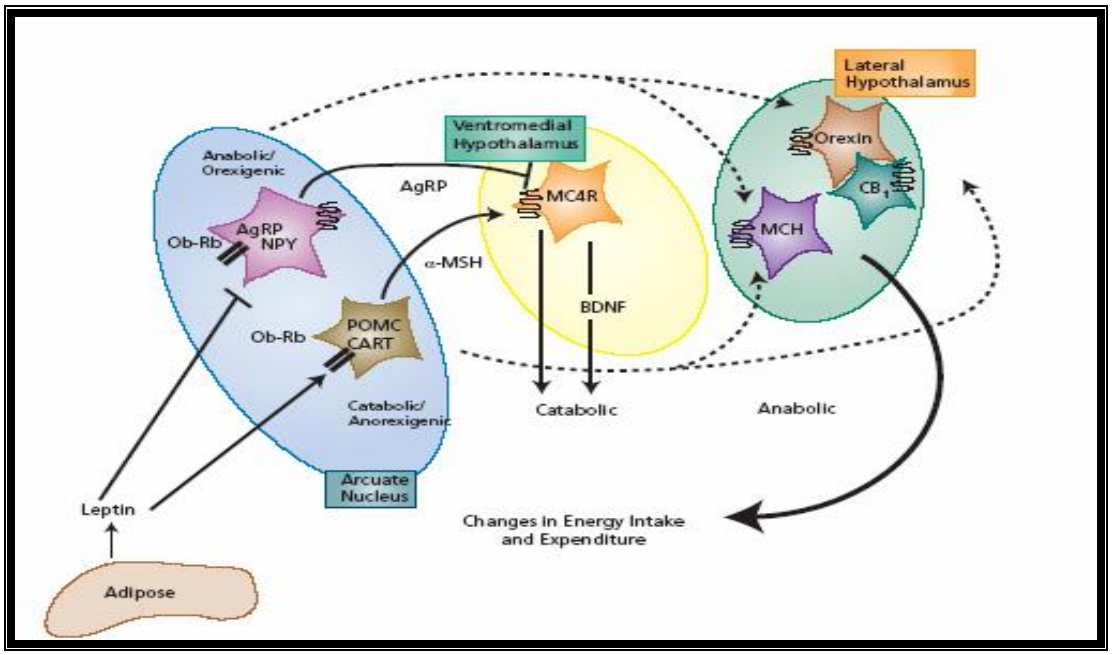

Figure 2: Leptin action and downstream effects in the arcuate nucleus, ventromedial hypothalamus and lateral hypothalamus(Nussey and Whiteheds, 2001). .

Once leptin has bound to the Ob-Rb receptor, it activates the stat3, which is phosphorylated and travels to the nucleus to, presumably, effect changes in gene expression. One of the main affects on gene expression is

the down-regulation of the expression of endocannabinoids, responsible for increasing appetite. There are other intracellular pathways activated by leptin, but less is known about how they function in this system. In response to leptin, receptor neurons have been shown to remodel themselves, changing the number and types of synapses that fire onto them **(**Margetic et al., 2002).

2.7.3 Leptin, obesity and diabetes

Although leptin is a circulating signal that reduces appetite, in general, obese people have an unusually high circulating concentration of leptin. (Considine et al., 1996). These people are resistant to the effects of leptin. Leptin could be regulated by insulin (Susan, 2000). The high sustained concentrations of leptin form the enlarged adipose stores result in leptin desensitization. The pathway of leptin control in obese people might be flawed at some point so the body doesn't adequately receive that satiety feeling subsequently to eating (Considine et al., 1996).

Disrupting leptin's appetite-controlling passageways leads to disturbance in two different brain body pathways: 1) pathway responsible for controlling appetite and fat storage which leads to increase eating and fat storage and 2) pathway responsible for telling the liver what to do with its stored glucose. Impairment of these two pathways leads to obesity, and obesity is known to significantly raise the risk of diabetes. However, it may take disruptions to both pathways to bring on full-blown diabetes and overwhelm the body's ability to control blood glucose levels via the action of insulin (American Diabetes Association 2005).

To determine whether leptin secretion is impaired in diabetes, Liu et al., (1999) compared basal and stimulated plasma leptin levels in diabetic subjects and healthy controls. Blood samples for assay of leptin and other hormones were obtained at baseline in 54 diabetic patients and 65 controls, at 8 hours, 16 hours, and 40 hours following ingestion of dexamethasone (4 mg) in 6 healthy and 12 controls. C-peptide status was defined as "negative" if < or =0.1 ng/mL or "positive" if > or =0.3 ng/mL, in fasting plasma. Basal plasma leptin levels were 19.7±2.2 ng/mL in nondiabetic subjects, 13.4±1.5 ng/ml in

C-peptide negative (n = 28) and 26.1±3.7 ng/mL in C-peptide positive (n = 26, p = 0.001) diabetic patients. Dexamethasone increased leptin levels of controls (n = 6) to 145±17% of baseline values at 8 hours (p = 0.03), 224±18% at 16 hours (p = 0.01), and 134±18% at 40 hours (p=0.05). The corresponding changes were 108±13%, 126±23%, and 98±16% in C-peptide negative (n=6), and 121±10%, 144±16% (p=0.03), and 147±23% (p=0.11) in C-peptide positive (n = 6) diabetic patients, respectively. The peak stimulated leptin levels were lower in the diabetic patients, compared with controls. Plasma insulin increased (p = 0.02) in controls, but not in the diabetic patients, following dexamethasone.

Tatti et al., (2001) compared the leptin concentration, and its relationship with some anthropometric and biochemical parameters related to insulin resistance in 140 moderately obese type 2 diabetics and 160 age and weight matched non-diabetic controls. The leptin levels were lower in the diabetic population only when both sexes were combined ($p < 0.05$) and were higher in the females of both groups. Among the nondiabetic, the leptin levels appeared to be related to BMI, %FM, HDL and FPI, while this was not the case in the diabetics. After correction for BMI, leptin appeared to be correlated with the FPI levels only in the non-diabetic females. When plasma leptin was included in a multiple linear regression model with plasma leptin as a dependent variable, BMI, W:Hr and FPI levels were significantly related to leptin in the non diabetic population, while no relationship reached the level of statistical significance among the diabetics, with the exception of the borderline value for the FPI (p = .052).

The possible associations between leptin and different clinical and biochemical characteristics of a large group of subjects with type 2 diabetes mellitus in Sudan were investigated (Abdelgadir, 2002). A total of 104 (45 men and 59 women) consecutive type 2 diabetes patients and 75 control subjects (34 men and 41 women) were studied. Leptin was higher in females than in males and correlated significantly to BMI. The main novel finding was that serum leptin was significantly lower in diabetic subjects compared with controls in both females (P=0.0001) and males (P=0.019), although BMI did

not differ between diabetic and nondiabetic subjects. Diabetic subjects treated with sulphonylurea (n=81) had lower BMI than those treated with diet alone or other hypoglycemic drugs (n=23) (P=0.0017), but there was no difference in leptin levels between the 2 groups after adjustment for BMI (P=0.87). In diabetic subjects, serum leptin correlated positively with the homeostatic assessment (HOMA) of both [beta]-cell function (P=0.018) and insulin resistance (P=0.038), whereas in control subjects, leptin correlated with insulin resistance (P=0.0016), but not with [beta]-cell function. Diabetic subjects had higher proinsulin levels (P=0.0031) and higher proinsulin to insulin ratio (P=0.0003) than nondiabetic subjects. In univariate analysis, proinsulin showed a weak correlation to leptin (P=0.049). In conclusion, it was shown in a large cohort of Sudanese subjects with type 2 diabetes that circulating leptin levels are lower in diabetic subjects than in controls of similar age and BMI.

Abu sayeed et al. (2003) reported that although leptin levels are increased in obesity, obese subjects with type 2 diabetes display reduced leptin. They examined whether leptin levels are also reduced in lean subjects with type 2 diabetes. Fifty nonobese Bangladeshi women with type 2 diabetes (aged 37.2±1.3 years) were selected randomly from the Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) outpatient department (28 were on diet and exercise, and 22 were on oral hypoglycemic agents; HbA1c 9.6 _ 0.8%). A total of 50 nondiabetic age- and BMI-matched health professional women (aged 33.4±1.9 years) served as control subjects. Circulating leptin, BMI, waist-to-hip ratio (WHR), and mid-arm circumference (MAC) were measured. A 75-g oral glucose tolerance test (OGTT) was undertaken with measurements of glucose and insulin (radioimmunoassay). Diabetic subjects had lower leptin (11.1±1.6 vs. 16.2±1.9 ng/ml, P=0.001), higher WHR (0.86±0.02 vs. 0.84±0.01; P=0.034), and lower MAC (23.7±0.4 vs. 25.4±0.7 cm; P=0.001) than nondiabetic subjects, without any difference in BMI (22.8±0.4 vs. 23.0 ±0.6 kg/m^2). Leptin correlated to MAC (r=0.46, P=0.001) but not to WHR(r=0.01). Although fasting insulin did not differ between the groups (84.2±16.6 vs. 92.7± 34.2 pmol/l), the 60-min insulin levels during the OGTT

were lower in the diabetic subjects (209±22 vs. 467±38 pmol/l, P=0.001) in spite of higher 60-min glucose levels in the diabetic subjects (14.2±4.8 vs. 6.8±2.1 mmol/l; P=0.001). In the diabetic subjects, leptin correlated significantly to fasting insulin independent of BMI (r=0.65, P=0.007).

Zabut et al, (2007) conducted their study to ascertain whether protohormone leptin and soluble leptin receptor (OB-Re) are correlated with BMI, gender, serum lipid profiles among adult individuals in the GS . Case group consisted of 83 adult individuals (BMI ≥25 kg/m^2) without history of other diseases and control group consisted of 83 eligible normal weight adult individuals (BMI 18.5-24.9 Kg/m^2). The results showed a significant positive correlation between BMI and leptin hormone among the case individuals (r=0.64, P<0.01). In contrast, OB-Re has inverse statistical relationship with BMI for the same individuals (r=-0.26, p=0.02). Surprisingly, there was no significant correlation between OB-Re and leptin among the case individuals (r=-0.16, p=0.14). For the case individuals, the leptin was also significantly higher (p=0.00) for the females (mean=72.40 ng/ml) than for the males (mean=44.05ng/ml). On the other hand, for the same individuals, OB-Re was slightly higher for the females (mean=9.75 ng/ml) than for the males (mean= 8.91 ng/ml) which was not statistically significant. Serum leptin, cholesterol, triglyceride and LDL-c levels were increased with increasing BMI. Conversely OB-Re and HDL-c were decreased with increasing BMI.

Welsh et al., (2008) related baseline leptin levels to CVD events (n=864) and incident diabetes (n=289) in an elderly population (n=5,672) over 3.2 years of follow-up. In treatment-, age-, and country-adjusted models, leptin was not associated with risk of CVD in men (hazard ratio 1.02 [95% CI 0.90–1.16] per unit log-leptin increase) or women (1.05 [0.91–1.20]) but was associated with risk of diabetes in men (2.75 [2.14–3.52]) and women (1.54 [1.22–1.94]). After adjusting for classic risk factors and BMI, C-reactive protein, and glucose, the diabetes association retained significance in men (1.85 [1.30–2.63]) but not in women (0.89 [0.64–1.26]). In conclusions Leptin, similar to other markers of adiposity in general, is more strongly related to risk of diabetes than CVD in the elderly.

Chapter 3

Materials and Methods

3.1 Study design

The study design was cross sectional

3.2 Target population

The target population is type 2 diabetic female patients from Al Rimal diabetic clinic in the G.S.

3.3 Sample size

The Sample size was 81 type 2 diabetic patients' females and 74 healthy females as control sample.

3.4 Inclusion criteria

Age: 40-60 years old female

Blood pressure: Normotensive

3.5 Exclusion criteria

Age below 40 or >60 year

Blood pressure high

Pregnancy women

Insulin dependent patient

3.6 Sampling

A total of 81 urine and 81 blood samples were collected from type 2 diabetic females, who were previously diagnosed according to the current WHO diagnostic criteria for diabetes, (WHO, 2006). Patients were attending the diabetic clinic at Al Rimal central clinic in Gaza city which is representative for

the Gaza Governorate. Seventy four urine and 74 blood samples were also collected from healthy females who served as controls.

3.7 Ethical Consideration

Obtained the necessary approval to conduct the study from Helsinki committee in the G.S (Annex 1). The approval was issued on Jun 2009. Helsinki committee is an authorized professional body for giving permission to researchers to conduct their studies with ethical concern in the area. Two official letters of requests sent from the Islamic University of Gaza to Palestinian Ministry of Health to obtain approval to conduct the study in Al Rimal central clinic and laboratories (Annexes 2 and 3). The participants were given a full explanation about the purpose of the study and assurance about the confidentiality of the information and that the participation was optional.

3.8 Data collection

3.8.1 Questionnaire interview

A meeting interview used for filling in the questionnaire which designated for matching the study need. All interviews were conducted face to face by the researcher. The questionnaire (Annexes 4 and 5) was based on diabetic clinic questions at Al Rimal central clinic with some modifications (Al Rimal Medical Center, Gaza, Palestine 2006). During the study the interviewer explained to the participants any of the confused questions that were not clear to them. Most questions were the yes/no questions, which offer a dichotomous choice(Backestrom and Hursh-Cesar, 1981). A questionnaire was piloted with 10 patients. The questionnaire includes questions on the personal data (name, age, education, family history of diabetes and diet) and clinical data including duration of DM (only for patients) and the most important complications of diabetes (retinopathy, CVD and neuropathy

3.8.2 Body mass index

Body mass index was calculated as the ratio of body weight in Kg/height in meter square. People with BMI=18.5–24.9 were considered to have normal

weight, people with BMI=25.0–29.9 were classified overweight, people with BMI≥30.0 were considered obese (WHO, 2000).

3.8.3 Specimen collection and biochemical analysis

Blood and urine samples were collected from 81 type 2 diabetic patient and 74 controls. Fasting overnight venous blood sample (about 6 ml) was drawn by a well trained medical technologist into vacutainer tubes from each control and diabetic individual. The blood was left for a while without anticoagulant to allow blood to clot. Then serum samples were obtained by centrifugation at room temperature by Rotina 46 Hitachi centrifuge, Japan at 4000 rpm/10 minutes. Random urine samples were collected from both patients and controls for the determination of microalbuminuria and creatinine. The urine samples were centrifuged by the same way as serum to precipitate all the debrises. About 0.5 ml of urine was transferred to the autoanalyzer (Konelab 60 Chemistry Autoanalyzer, Finland) for the detection of microalbuminuria, Another part of urine was diluted 1/20 (25 urine/475 distilled water) for the determination of creatinine for estimation of albumin/creatinine ratio.

Serum glucose, urea, creatinine, cholesterol, triglycerides were analysed by Konelab 60 Autoanalyzer in Al Rimal Clinical Chemistry Laboratory. Quality assurance program was carried out by analyzing 2 levels of lyophilized multi control sera and 1 level of lyophilized urine control from Biosystems kit, Spain, on every run of analysis. For serum analysis we used deionized water as 1^{st} calibrator and multicalibrator (CALS) as 2^{nd} calibrator for the determination of colorimetric tests.

Calculation of colorimetric tests for glucose, urea, creatinine, cholesterol, triglycerides, were performed by the autoanalyzer automatically according to beer's law after calibration and adjustment of the photometers against water blank using a specific program of every test inserted to the instrument.

$$\text{The concentration of colorimetric test} = \frac{A_{Test} \times C_{CALS}}{A_{CALS}}$$

Serum high density lipoprotein cholesterol (HDL-C) was determined spectrophotometrically and then low density lipoprotein cholesterol (LDL-C) was determined. Urine samples were also analysed by Konelab 60 autoanalyzer.

3.8.3.1 Determination of serum glucose

Serum glucose was determined by glucose oxidase (GOD)/glucose peroxidase (POD) method (Trinder,1969) using Labkit Kit, Spain.

Principle

GOD catalyses the oxidation of glucose to gluconic acid. The formed hydrogen peroxide (H_20_2) is detected by a chromogenic oxygen acceptor, phenol-aminophenazone in the presence of POD.

B-D-Glucose + $2H_20 + O_2$ $\xrightarrow{GOD}$ Gluconic acid + H_20_2

H_2O_2 + Phenol + 4-Aminophenazone (4-AP) $\xrightarrow{POD}$ Quinone + H_2O

The intensity of the red color formed is proportional to glucose concentration in the sample.

Reagents

Reagent	Component	Concentration
Reagent 1	TRIS pH 74	92 mmol/L
	Phenol	0.3 mmol/L
	GOD	15000 U/L
	POD	1000 U/L
	4-AP	2.6 mmoVL

Procedure

About 0.5 ml of serum was transferred to the Konelab 60 Chemistry Autoanalyzer, to perform the test according to these parameters:

Parameter	Value
Reagent volume (µl)	140
Serum volume (µl)	2.0
Calibrator 1 (mg/dl)	0.0
Calibrator 2 CALS (mg/dl)	178
Incubation time (s)	240
Wavelength (nm)	510
Calibrator type	Linear
Measurement Type	End point

3.8.3.2 Determination of serum Urea

Serum urea was determined by urease - glutamate dehydrogenase (GDH)/UV method (Gutmann and Bergmeyer 1974) using BioSystems kit, Spain.

Principle

Urea in the sample is consumed, by means of the coupled reactions described below. The decrease of NADH can be measured photometrically at 340 nm.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_2$$

$$NH_4^+ + \text{NADH} + \text{2-oxoglutarate} \xrightarrow{\text{GDH}} \text{glutamate} + NAD^+$$

Reagents

Reagent	Component	Concentration
Reagent 1	TCis	100 mmol/L
	2-oxoglutarate	5.6 mmol/L
	Urease	> 140 U/mL
	GDH	> 140 U/mL
	ethylene glycol	220 g/L
	sodium azide	9.5 g/L
	pH	8.0
Reagent 2	NADH	1.5 mmol/L
	sodium azide	9.5 g/L

Procedure

About 0.5 ml of serum was transferred to the Konelab 60 Chemistry Autoanalyzer, to perform the test according to these parameters:

Parameter	Value
Reagent volume (µl)	140
Serum volume (µl)	2
Calibrator 1 (mg/dl)	0.0
Calibrator 2 CALS (mg/dl)	51
Incubation time (s)	60
Reading time (s)	60
Wavelength (nm)	340
Calibrator type	Linear
Measurement Type	Kinetic

3.8.3.3 Determination of creatinine

Serum creatinine was determined by Alkaline Picrate method (Fabiny and Ertingshausen, 1971) using BioSystems kit, Spain.

Principle

Creatinine in the sample reacts with picrate in alkaline medium forming a colored complex. The complex formation rate is measured in a short period to avoid interferences.

Reagents

Reagent	Component	Concentration
Reagent 1	Picric acid	25 mmol/L
Reagent 2	Sodium hydroxide Detergent	0.4 mol/L

Procedure

About 0.5 ml of serum was transferred to the Konelab 60 Chemistry Autoanalyzer, to perform the test according to these parameters:

Parameter	Value
Reagent volume (µl)	158
Serum volume (µl)	20
Calibrator 1 (mg/dl)	0.0
Calibrator 2 CALS (mg/dl)	2.71
Incubation time (s)	60
Reading time (s)	60
Wavelength (nm)	510
Calibrator type	Linear
Measurement Type	Kinetic

3.8.3.4 Determination of serum cholesterol

Serum cholesterol was determined by cholesterol oxidase (COD)/POD method (Meiatlini,et al, 1978) using BioSystems kit, Spain.

Principle

Free and esterified cholesterol in the sample originates, by means of the coupled reactions described below, a colored complex that can be measured photometrically.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + 1/2\ O_2 + H_2O \xrightarrow{\text{COD}} \text{Cholestenone} + H_2O_2$$

$$2\ H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4\ H_2O$$

Reagents

Reagent	Component	Concentration
Reagent 1	Pipes	35 mmol/L
	Sodium cholate	0.5 mmol/L
	Phenol	28 mmol/L
	Cholesterol esterase	> 0. 2 U/mL
	COD	> 0.1 U/mL
	POD	> 0.8 U/mL
	4-ammoantipyrine	0.5 mmol/L
	pH	7.0

Procedure

About 0.5 ml of serum was transferred to the Konelab 60 Chemistry Autoanalyzer, to perform the test according to these parameters:

Parameter	Value
Reagent volume (µl)	140
Serum volume (µl)	2
Calibrator 1 (mg/dl)	0.0
Calibrator 2 CALS (mg/dl)	236
Incubation time (s)	240
Wavelength (nm)	510
Calibrator type	Linear
Measurement Type	End point

3.8.3.5 Determination of serum triglycerides

Serum triglycerides were determined by Glycerol phosphate oxidase/peroxidase method (Bucolo and David,1973).using BioSystems kit, Spain.

Principle

Triglycerides in the sample orginates, by means of the coupled reactions described below colored complex that can be measured photometrically

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol - 3 - P} + \text{ADP}$$

$$\text{Glycerol - 3 -P} + O_2 \xrightarrow{\text{G-3-P-oxidase}} \text{Dihydroxyacetone - P} + H_2O$$

$$2H_2O_2 + \text{4- Aminoantipyrine} + \text{4-Chlorophenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_20$$

Reagents

Reagent	Component	Concentration
Reagent 1	Pipes	45 mmol/L
	Magnesium chloride	5 mmol/L
	4-chlorophenol	6 mmol/L
	Lipase	> 100 U/mL
	Glycerol kinase	> 1.5 U/mL
	Glycerol-3-phosphate oxidase	> 4 U/mL
	Peroxidase	> 0.8 U/mL
	A-aminoantipyrine	0.75 mmol/L
	ATP	0.9 mmol/L
	pH	7.0

Procedure

About 0.5 ml of serum was transferred to the Konelab 60 Chemistry Autoanalyzer, to perform the test according to these parameters:

Parameter	Value
Reagent volume (µl)	140
Serum volume (µl)	2
Calibrator 1 (mg/dl)	0.0
Calibrator 2 CALS (mg/dl)	157
Incubation time (s)	240
Wavelength (nm)	510
Calibrator type	Linear
Measurement Type	End point

3.8.3.6 Determination of serum high density lipoproteins (HDL-C)

HDL-C was determined by precipitating method (Grove, 1979) using Labkit kit, Spain.

Principle The VLDL and LDL-C from serum or plasma are precipitated by phosphotungstate in the presence of magnesium ions. After removed by centrifugation the clear supernatant is containing high density lipoproteins (HDL-C) and used for the determination of it**.**

Reagents

Reagent	Component	Concentration
Reagent 1	Phosphotungstic acid Magnesium chloride	14 mmol/L 2 mmol/L

Procedure

1. Pipette into a centrifuge tube 25 µl of HDL-C reagent and 250 µl serum.

Mix well. Allow to stand for 10 minutes at room temperature.

2. Centrifuge at 4000 rpm for 10 minutes. Collect the supernatant and test HDL-C.

3. Pipette into centrifuge tube 1 ml cholesterol reagent and 10 µl of the supernatant. Mix well. Allow to stand for 10 minutes at room temperature.

4. Set the Unicam spectrophotometer, United Kingdom, at 505 nm and adjust it to zero with blank reagent. Read the Absorbance (A) of the test, and standard against reagent blank.

Calculation:

$$\text{HDL Concentration} = \frac{(A)\ \text{Test} \times (C)\ \text{Standard}}{(A)\ \text{Standard}}$$

3.8.3.7 Determination of serum low density lipoproteins LDL-C

LDL-C can be calculated using the empirical relationship of (Friedewald, et al 1972)

Principle

The ultracentrifugal measurement of LDL-C is time consuming and expensive and requires special equipment. For this reason, LDL-C is most commonly estimated from quantitative measurements of total and HDL-cholesterol and plasma triglycerides (TG) using the empirical relationship of Friedewald.

The Equation

LDL-C = Total Cholesterol - HDL-C - TG/5

3.8.3.8 Determination of serum leptin

Determination of human serum leptin level was carried out by competitive enzyme immunoassay (Diagnostic System Laboratories (DSL). USA) technique

Principle

The DSL-10-23100 ACTIVE Human Leptin ELISA is an enzymatically amplified "two-step" sandwich-type immunoassay. In the assay, Standards, Controls and unknown serum or plasma samples were incubated in microtitration wells, which have been coated with anti-human leptin antibody. After incubation and washing, the wells were treated with another anti-human leptin detection antibody labeled with the enzyme horseradish peroxidase (HRP). After a second incubation and washing step, the wells were incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution was then added and the degree of enzymatic turnover of the substrate was determined by dual wavelength absorbance measurement at 450 and 620 nm. The absorbance measured was directly proportional to the concentration of human leptin present. A set of human leptin standards was used to plot a standard

curve of absorbance versus human leptin concentration from which the human leptin concentrations in the sample can be calculated.

The assay procedure sheets were available with the kit, the application of assay procedure mentioned below.

Assay procedure

Annabel all specimens and reagents to reach room temperature (~25°C) and mix thoroughly by gentle inversion before use. Standards, Controls and samples should be assayed in duplicate.

1. The microtitration strips were marked to be used.

2. Twenty five microlitters of the standards, controls and samples were Pipeted into the appropriate wells.

3. One hundred Microlitters of the Assay Buffer E were added to each well using a semi-automatic dispenser.

4. Incubate the wells, shaking at a fast speed (500-700 rpm) on an orbital microplate shaker, at room temperature (~25 °C) for 2 hours.

5. Aspirate and wash each well 5 times with the Wash Solution using an automatic microplate washer. Blot dry by inverting plate on absorbent material.

6. The Antibody-Enzyme Conjugate Solution was prepared by diluting the Antibody-Enzyme Conjugate Concentrate in the Assay Buffer.

7. One hundred microlitters of the Antibody-Enzyme Conjugate Solution was added to each well using a semi-automatic dispenser.

8. The wells were incubated, shaked at a fast speed (500-700 rpm) on an orbital microplate shaker, at room temperature (~25 °C) for 1 hour.

9. Aspirate and wash each well 5 times with the Wash Solution using an automatic microplate washer. Blot dry by inverting plate on absorbent material.

10. One hundred Microlitters of the TMB Chromogen Solution was added to each well using a semi-automatic dispenser.

11. Incubate the wells, shaking at a fast speed (500-700 rpm) on an orbital microplate shaker, at room temperature (~25°C) for 10 minutes. Avoid exposure to direct sunlight.

12. One hundred Microlitters of the Stopping Solution (0.2M sulfuric acid) was added to each well using a semi-automatic dispenser.

13. The absorbance of the solution in the wells was read within 30 minutes, using a microplate reader set to 450 nm.

Calculation

A. The mean absorbance for each standard, control and samples were calculated.

B. Plot the log of the human leptin concentrations in ng/mL along the x-axis versus the mean absorbance readings for each of the standards along the y-axis versus, using a linear curve-fit. Alternatively, the data can be plotted linear vs. linear and a smoothed spine curve-fit can be used.

C. Determine the human leptin concentrations of the controls and samples from the standard curve by matching their mean absorbance readings with the corresponding human leptin concentrations.

3.8.3.9 Determination of urine microalbuminuria

Urine microalbuminuria was determined by Immunoturbidometry-Latex method (Harmoinen, et al, 1985) using BioSystems kit, Spain.

Principle

Albumin in the urine sample causes agglutination of the latex particles coated with anti-human albumin. The increase of the particles agglutination is proportional to the albumin concentration and can be measured immunoturbidometrically.

Reagents

Reagent	Component	Concentration
Reagent 1	Borate buffer sodium azide pH	0.1 mol/L 0.95 g/L 10.0
Reagent 2	Suspension of latex particles coated with anti-human albumin antibodies Sodium azide	 0.95 g/L
Albumin	Human albumin	47mg/L

Procedure

About 0.5 ml of serum was transferred to the Konelab 60 Chemistry Autoanalyzer, to perform the test according to these parameters:

Parameter	Value
Reagent volume (µl)	200
Serum volume (µl)	2
Calibrator 1 (mg/L)	0.0
Calibrator 2 Alb (mg/L)	47
Incubation time (s)	130
Wavelength (nm)	540
Calibrator type	Linear
Measurement Type	End point

3.9 Data analysis

Data were analyzed using Statistical Package of Social Sciences (SPSS) system (version 13.0). The following statistical tests were applied:

- Frequency distributions
- Chi – Square Test
- Analysis of variance (ANOVA)
- Independent-samples *t*-test
- Pearson's correlation test

The percentage difference was calculated according to the formula:

Percentage difference= mean of patient – mean of control / mean of control x 100.

Probability values (p) were obtained from the student's table of '*t*' and significance was *at* $p < 0.05$. Range as minimum and maximum values was used.

Chapter 4

Results

4.1 General characteristics of the study population

The present study is a cross sectional included 155 females (74 controls and 81 type 2 diabetics). The average age of the controls was 48.0±5.8 years whereas that of diabetic patients was 52.2±6.1 years (Table 1). Analysis of the educational status of the controls and patients showed that 16 (21.6%) and 2 (2.5%) had diploma or university degree, 29 (39.2%) and 33 (40.7%) had finished secondary school, 15 (20.3%) and 22 (27.2%) had finished preparatory school, 10 (13.5%) and 16 (19.8%) had passed primary school, and 4 (5.4%) and 8 (9.9%) were illiterate, respectively. Regarding family history, 40 (54.1%) controls and 57 (70.4%) patients reported that they have a family history of diabetes. In addition, the number of controls and patients on diet were 7 (9.5%) and 56 (69.1%), respectively.

Table 1: General characteristics of the study population (n=155)

General characteristics	**Controls (n=74)**	**Diabetics (n=81)**
Mean age (Year)	48.0±5.8	52.2±6.1
Education	**n (%)**	**n (%)**
Illiterate	4 (5.4)	8 (9.9)
Primary school	10 (13.5)	16 (19.8)
Preparatory school	15 (20.3)	22 (27.2)
Secondary school	29 (39.2)	33 (40.7)
Diploma or University	16 (21.6)	2 (2.5)
Family history		
Yes	40 (54.1)	57(70.4)
No	34 (45.9)	24 (29.6)
Diet		
Yes	7 (9.5)	56 (69.1)
No	67(90.5)	25(30.9)

Table 2 shows the association between diabetes, educational level, family history and diet. There was a progressive decrease in the mean blood glucose level with increasing the patient educational level. This relationship was statistically significant (F_{ANOVA}=2.82, P=0.027). Regarding family history, diabetes was found to be associated with family history (χ^2=3.20, P=0.05). Also, there was a significant slightly association between diabetes and going in diet (χ^2=95.93, P=0.000).

Table 2: Association between diabetes and general characteristics of the study population including educational level, family history and diet

General characteristics	Blood glucose level (mg/dl)
Educational level (patients)	**Mean±SD**
Illiterate	180.1±132.1
Primary school	166.8±85.7
Preparatory school	161.0±87.1
Secondary school	144.3±67.9
Diploma or University	99.3±28.9
	(F_{ANOVA}= 2.82, P=0.027)
Family history	**Adopted family history**
	n (%)
Patients	57 (69.5)
Controls	40 (55.6)
	(χ^2 = 3.20, P=0.05)
Diet	**On diet**
	n (%)
Patients	56 (68.3)
Control	5 (6.9)
	(χ^2 =95.93, P=0.000)

4.2 Distribution of diabetic patients by diabetes duration

Table 3 shows that patients with diabetic since less than 5 years were 55 (55.5%), whereas those with diabetic duration of 5-10 years were 22 (27.2%). The rest of patients 14 (17.3%) had diabetes for more than 10 years.

Table 3: Distribution of diabetic patients (n=81) by diabetes duration

Duration of diabetes (Year)	**No.**	**%**
< 5	45	55.5
5-10	22	27.2
>10	14	17.3

4.3 self-reported complications

Table 4 summarizes the main self-reported complications among the patients and the controls. The percentages of retinopathy, CVD and neuropathy were higher in diabetic patients compared to the controls (28.4, 4.9 and 19.8% vs. 1.4, 0.0 and 2.7%, p=0.001).

Table 4. The main self-reported complications among the study population (n=155)

Complication	**Control** (n=74) **n (%)**	**Patients** (n=81) **n (%)**	c^2	**P-Value ***
Retinopathy	1 (1.4)	23 (28.4)	25.42	0.001
CVD**	0(0)	4 (4.9)	-	-
Neuropathy	2 (2.7)	16 (19.8)	11.86	0.001

* P- value for chi – Square Test, CVD**: Cardiovascular diseases

The main self-reported complications and their relation with duration of diabetes is demonstrated in Table 5. In general, the longer the duration of diabetes mellitus, the higher the percentage of self reported complications among patients. This positive relationship was significant for retinopathy and neuropathy (p=0.023 and 0.030, respectively).

Table 5. The main self-reported complications and their relation to duration of diabetes

Complication	**Duration of diabetes (Year)**			c^2	**P-Value ***
	< 5 (n=45) **n (%)**	**5-10** (n=22) **n (%)**	**> 10** (n=14) **n (%)**		
Retinopathy	8 (17.8)	10 (45.5)	5 (35.7)	7.29	0.023
CVD**	2 (4.4)	1 (4.5)	1 (7.1)	0.160	0.923
Neuropathy	5 (11.1)	5 (22.7)	6 (42.9)	6.41	0.030

* P- value for chi – Square Test, CVD**: Cardiovascular diseases

4.4 Body mass index

The BMI body of diabetic patients and controls was illustrated in Table 6. The numbers of normal, overweight and obese patients were 5 (6.2%), 26 (32.1%) and 50 (61.7%) whereas in controls they were 14 (18.9%), 18 (24.3) and 42 (56.8). P- value for chi – Square Test showed significant association between normal versus overweight versus obese among controls and patients (χ^2=6.11, P=0.047).

Table 6. Body mass index of the study population (n=155)

BMI*	**Control** (n=74) **n (%)**	**Patients** (n=81) **n (%)**	**P value**
Normal	14 (18.9)	5 (6.2)	χ^2 =6.11 P =0.047
Overweight	18 (24.3)	26 (32.1)	
Obese	42 (56.8)	50 (61.7)	

* People with BMI=18.5–24.9 were considered to have normal weight, people with BMI=25.0–29.9 were classified overweight, people with BMI≥30.0 were considered obese (WHO, 2000).

4.5 Biochemical analysis

4.5.1 Serum glucose among diabetics and controls

Table 7 points out that the mean serum glucose level in patients was significantly higher than that in controls (197.6±81.6 vs. 89.9+14.2, % difference=119.8 and p=0.000).

Table 7: Serum glucose of the study population (n=155)

Parameter	**Controls** mean±SD	**Diabetics** mean±SD	**% difference**	**t**	**P-value**
Serum glucose (mg/dl) Range (min – max)	89.9±14.2 (60 – 148)	197.6±81.6 (74 – 476)	119.8	10.149	0.000

Reference range=70-115 mg/dl (Thomus, 1998).

4.5.2 Serum urea and creatinine of diabetics and controls

As illustrated in Table 8, the mean serum urea concentration was slightly decreased in diabetics compared to controls showing percentage difference of 3.6 (24.0±7.9 vs. 24.9±7.8). However, this change was not significant

(p=0.492). In contrast, serum creatinine concentration was slightly increased in diabetics compared to controls (0.73±0.23 vs. 0.70±0.16, % difference=4.3). This change was not also significant (p=0.257)

Table 8: Serum urea and creatinine of the study population (n=155)

Parameter (mg/dl)	**Controls** mean±SD	**Diabetics** mean±SD	**% difference**	**t**	**P-value**
Urea Range (min – max)	24.9±7.8 (13 –60)	24.0±7.9 (12 –51)	-3.6	-0.688	0.492
Creatinine Range (min – max)	0.70±0.16 (0.3 –1.1)	0.73±0.23 (0.3 –1.6)	4.3	1.138	0.257

Reference range: Urea=15-43 mg/dl (Thomus, 1998) and creatinine=0.6-1.1 mg/dl (Newman and Price, 1999).

4.5.3 Lipid profile of diabetics and controls

The tested lipid profile was cholesterol, triglycerides, low density lipoprotein cholesterol and high density lipoprotein cholesterol**.** As depicted from Table 9, the average levels of serum cholesterol, TG and LDL-C were significantly higher in diabetics (204.3±52.6mg/dl, 187.7±103.2mg/dl and 105.9±35.2mg/dl) compared to controls (180.5±36.1 mg/dl, 118.3±72.7 mg/dl and 91.9±31.5 mg/dl) with percentage differences of 13.2, 58.7 and 15.2%, p=0.002, p=0.000 and p=0.011, respectively. In contrast, HDL-C was significantly lower in diabetics than in controls (43.1±13.1 mg/dl vs. 47.9±16.6 mg/dl, % difference=10.0 and p=0.045).

Table 9: Serum cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) of the study population (n=155)

Lipid Profile (mg/dl)	**Controls** mean±SD	**Diabetics** mean±SD	**% difference**	**t**	**P-value**
Cholesterol Range (min – max)	180.5±36.1 (93-267)	204.3±52.6 (89-413)	13.2	3.221	0.002
Triglycerides Range (min – max)	118.3±72.7 (29-427)	187.7±103.2 (43-800)	58.7	4.759	0.000
HDL-C Range (min – max)	47.9±16.6 (24-105)	43.1±13.1 (21-96)	-10.0	2.020	0.045
LDL-C Range (min – max)	91.9±31.5 (11-163)	105.9±35.2 (19-183)	15.2	2.562	0.011

Reference range: cholesterol≤200 mg/dl (Schaefer and McNamara, 1997), triglyceride<200 mg/dl (Cole et al., 1997), HDL-C=45-65 mg/dl (Grove, 1979) and LDL-C<150 mg/dl (Friedewald et al., 1972).

4.5.4 Serum leptin of diabetics and controls

Table 10 shows the average serum leptin level in controls and diabetic patients. There was a significant decrease in the mean level of leptin in diabetics compared to controls (12.3±8.7 ng/ml vs. 16.8±14.4 ng/ml, % difference=26.8 and p=0.018).

Table 10: Serum leptin of the study population (n=155)

Parameter	**Controls** mean±SD	**Diabetics** mean±SD	**% difference**	**t**	**P-value**
Serum leptin (ng/ml) Range (min – max)	16.8±14.4 (2.5 - 82.9)	12.3±8.7 (2.1 – 40.8)	-26.8	2.389	0.018

Reference range: 7.5-58.7 ng/ml (Considine et al., 1996).

4.6 Leptin relations

4.6.1 Leptin relation to glucose

The correlation between leptin and glucose levels is illustrated in Table 11. The Pearson correlation test showed that the higher the glucose level, the lower the level of leptin. This negative correlation was significant and confirmed the association of diabetes with leptin (r=-0.170, p=0.030)

Table 11. The correlation between leptin and glucose levels of the study population (n=155)

		Glucose	**Leptin**
Glucose	Pearson's correlation	1	-0.176*
	Sig. (2-tailed)		0.030
Leptin	Pearson's correlation	-0.176*	1
	Sig. (2-tailed)	0.030	

* Correlation is significant at the 0.05 level (2-tailed).

4.6.2 Leptin relation to diet and BMI

The association of leptin with BMI and diet of the study population is presented in Table 12. The t-test showed that the individuals who were not restricted to diet had higher leptin levels than those who did (t=1.66, p=0.07). Regarding BMI, ANOVA test revealed that the larger the BMI, the higher the level of leptin. This positive relationship was statistically significant (F=4.45, p=0.013)

Table 12. The association of leptin with BMI and diet of the study population (n=155)

Parameter	Leptin Level ng/ml Mean + SD	P value
Diet		
Yes	12.49+9.02	t= 1.66
No	15.76+13.37	P= 0.07
BMI*		
Normal	9.92+7.5	F =4.45
Overweight	11.35+10.4	P =0.013
Obese	16.7+11.7	

* People with BMI=18.5–24.9 were considered to have normal weight, people with BMI=25.0–29.9 were classified overweight, people with BMI≥30.0 were considered obese (WHO, 2000).

4.6.3 Leptin relation to lipid profile

The correlation between leptin and lipid profile of the study population is pointed out in Table 13. The Pearson correlation test showed that with decreasing leptin levels there are increases in cholesterol, triglyceride and LDL levels. Such negative correlation was significant for trigelycerides (r=-0.174, p=0.032) and not significant for cholesterol and LDL (r=-0.120, p=0.139 and -0.084 and p=0.305, respectively). On the other hand, there is a positive correlation between leptin level and HDL-C and this correlation was statistically significant (r=-0.200, p=0.013).

Table 13: The correlation between leptin and lipid profil of the study population.

Lipid Profile (mg/dl)	Leptin	
	Pearson correlation (r)	P-value
Cholesterol	-0.120	0.139
Triglycerides	-0.174	0.032
HDL-C	0.200	0.013
LDL-C	-0.084	0.305

4.7 Urine analysis

The averages of urine albumin, creatinine concentrations and albumin/creatinine ratio of controls and diabetic patients are presented in Table 14. Diabetic patients showed higher albumin levels in their urine compared to controls (41.1±62.6μg/dl vs. 24.7±23.5μg/dl, % difference=66.4 and p=0.045. On the other hand, as in serum, there was no statistically significant difference in the mean levels of urinary creatinine in diabetics and controls (75.1±40.3 mg/dl vs. 80.0±49.8 mg/dl, % difference=6.1 and p=0.547). However, when albumin/creatinine ratio was calculated and averaged, significant increase was observed between diabetic and controls (0.72±1.11μg/mg vs. 0.37±0.41μg/mg, % difference=94.6 and p=0.017).

Table 14: Urine albumin, creatinine and albumin/creatinine ratio of the study population (n=155)

Parameter	Controls mean±SD	Diabetics mean±SD	% difference	t	P-value
Albumin (μg/dl) Range (min – max)	24.7±23.5 (1 – 133)	41.1±62.6 (4.3 – 400)	66.4	2.029	0.045
Creatinine (mg/dl) Range (min – max)	80.0±49.8 (5 – 244)	75.1±40.3 (20 – 206)	6.1	0.604	0.547
albumin/creatinine ratio (μg/mg) Range (min – max)	0.37±0.41 (1.5-253)	0.72±1.11 (4.2-527)	94.6	2.414	0.017

Reference range: microalbuminuria: urinary albumin 30-300 mg/24hr, urinary creatinine=11-312 mg/dl and albumin/creatinine ratio<0.34 μg/mg (Rulan et al., 2007).

Table 15 showed microalbuminurea among controls and diabetic patients. Microalbuminuric controls and patients were 7 (9.5%) and 20 (24.7%), repectively. P- value for chi – Square Test showed significant association between microalbuminuric versus normoalbuminuric controls and patients (χ^2=3.59, p=0.047).

Table 15. Microalbuminuria among the study population (n=155)

Condition	Control (n=74) No. (%)	Patients (n=81) No. (%)	P- value
Microalbuminuric	7 (9.5)	20 (24.7)	χ^2 =3.59
Normoalbuminuric	67 (90.5)	61 (75.3)	P =0.047

*Microalbuminuria: urinary albumin 30-300 mg/24hr.

Chapter 5

Discussion

Although diabetes mellitus is prevalent in the GS, there are under-diagnosis and under-reporting of the disease. Data were limited to annual reports emerged from the PMOH. Follow up patients in Gaza hospitals and clinics are only restricted to monitoring blood glucose level when the patient visits the clinic. Just recently (in the last two years), few studies have been carried out on microalbuminurea and other early markers for diabetic nephropathy among type 2 diabetic patients in the GS (Tibi, 2007, Shubair, 2008 and Abu Hilal, 2009). However, the role of leptin in such studies was not speculated. In another study, leptin was investigated in nondiabetic obese patients (Al-Holi, 2006). Therefore, the present study was the first to demonstrate leptin level and its relation to blood glucose levels as well as to some blood biochemical parameters among diabetic females in Gaza City, GS.

In addition to the assessment of leptin, the contributing risk factors as well as some biochemical features of type 2 diabetes the females were investigated and compared with those of controls. Understanding the role of leptin in diabetes as well as identifying risk factors and metabolic alterations will no doubt be helpful in the management and control of the disease.

The target population was 81 type 2 diabetic females from Al Remal diabetic clinic which is the representative clinic for diabetic patients in Gaza Governorate. Their mean age was 52.2±6.1 years. It was reported that type 2 diabetes mellitus usually develops after age 40 years (Rodger, 1991 and Umpierrez et al., 2006).

Data presented here showed progressive decrease in the mean blood glucose level with increasing the patient educational level. This implies that the well educated patients had a better control of the disease through adoption of healthy diets i.e. their knowledge is reflected in good practice. Diabetes was found to be associated with family history. This finding is in agreement with other studies who reported that family history is a risk factor

for diabetes (Annis et al., 2002 and Pijl et al., 2009). Although there was a significant association between diabetes and diet, sill around third of the patients did not go on diet. This necessitates launching of educational programs to show the importance of diet in controlling the disease.

The finding that more than half of patients (55.5%) had diabetics since less than 5 years do confirm the idea that type 2 diabetes has long asymptomatic pre-clinical phase which frequently goes undetected. At the time of diagnosis, the patient could have one or more diabetes complications (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2003). Distribution of self reported diabetic complications among our diabetic patients confirmed this view. However, this point still needs further investigation.

As indicated in the results, the most self reported symptoms among diabetic patients were retinopathy, neuropathy and cardiovascular diseases. The prevalence of such symptoms were positively associated with the progress of the disease i.e. the longer the duration of diabetes mellitus, the higher the percentage of self reported complications among patients. Several studies reported similar diabetic complications with increasing rates upon disease progress (Savage, 1996, Dyck et al., 2002, Marshall, 2006 and The National Eye Institute, 2006).

BMI provides a reliable indicator of body fatness for most people and it is used to screen for weight categories that may lead to health problems (CDC, 2007). Therefore, obesity is commonly defined as a BMI of 30 kg/m^2 or higher. This definition distinguishes obesity from being pre-obese or overweight, which is classified as a BMI of 25 kg/m^2 but less than 30 kg/m^2 (WHO, 2000). In the study, BMI was significantly associated with diabetes, where most of diabetic patients were obese. In other words, obese individuals are at higher risk for diabetes. However, about half of control individuals in our study sample were also obese. This may explain the border line significance of p value (p=0.047) on comparison the numbers and percentages of controls and patients.

The literature supported the results in that obesity is a major risk factor for chronic diseases including diabetes (El-Hazmi et al., 1997, Marshall, 2006 and Yassin et al., 2009). About 55 percent of type 2 diabetics are obese (Eberhart et al., 2004). Chronic obesity leads to increased insulin resistance that can develop into diabetes, most likely because adipose tissue is a source of several chemical signals to other tissues. Other research showed that type 2 diabetes causes obesity as an effect of the changes in metabolism and other deranged cell behavior attendant on insulin resistance (Camastra, 1999).

Serum glucose levels were significantly higher in diabetic patients than controls, although diabetic patients were under medication. This may be explained in part by the finding that about third of diabetic patients did not compliance with diet regime. In addition, medication regime may be not followed. High-fat diet was found to increase risk of type 2 diabetes (Lovejoy, 2002). In a previous study to assess non-compliance among 216 type 2 diabetic patients in GS, (Zakout 2006) reported that three quarters of the study population had poor glycemic control and half of them were not compliance with the medication regime. Typical reasons cited by patients included forgetfulness, frustration, feeling better without treatment, polypharmacy, fear from drug side-effect and unavailability of drugs.

As indicated in the result data serum urea and creatinine concentrations of diabetics were not significantly changed compared to that of controls. Urea is formed by the liver as an end product of protein breakdown and creatinine is a waste product that is normally filtered from the blood and excreted with the urine. Creatinine and urea are markers of the kidney function and changes in their levels are indicating renal diseases (Debra Manzella, 2008). Therefore, the results indicated that many of the studied patients are still in the early stages of the disease. This was obvious in the finding that more than half of patients (55.5%) had diabetics since less than 5 years. However, it was difficult to determine the onset of such changes in urea and creatinine concentrations and this may lead to controversial results (Varghese et al., 2001 and El Meligi et al., 2003). Therefore, the creatinine

levels must be watched carefully to determine how much function the kidneys have and this does vary slightly.

In the present results there were significant increases in the levels of total cholesterol, triglycerides and LDL-C in diabetics when compared to controls. In contrast there was a significant decrease in HDL-C level in diabetics. It was known that cholesterol, TAG and LDL-C are elevated in diabetic patients (Barrett-Connor et al., 1982 and El Meligi et al., 2003). The abnormal high concentrations of serum lipids in diabetics is due, mainly to increase in the mobilization of free fatty acids from fat depots, since insulin inhibits the hormone sensitive lipase. Excess fatty acids in serum of diabetics are converted into TG, phospholipids and cholesterol in liver. These three substances with protein may be discharged into blood in the form of lipoproteins (Taskinen, 1992 and Jaworski et al., 2007)

Leptin is a new hormone influencing food intake, energy expenditure and body weight (Prieur et al., 2008). This hormone is produced by adipocytes, exerts its effects on brain, endocrine pancreas and other organs by activating transmembrane receptors and is cleared from plasma mainly by the kidney (Chabova et al., 1999). The results of study revealed that mean serum leptin was significantly decreased in diabetic patients compared to controls The Pearson correlation test showed that the higher the glucose level, the lower the level of leptin. This negative correlation was significant and confirmed the association of diabetes with leptin ($r= -0.176$, $p = 0.030$).

Several studies support such finding and others contradict it. To determine whether leptin secretion is impaired in diabetes, Liu et al., (1999) compared basal plasma leptin levels in diabetic subjects (n=54) and healthy controls (n=65). They found that basal plasma leptin levels were 19.7±2.2 ng/mL in nondiabetic subjects compared to 13.4±1.5 ng/ml in diabetics which favours our finding. In another study, Tatti et al., (2001) compared the leptin concentration, and its relationship with some anthropometric and biochemical parameters related to insulin resistance in 140 moderately obese type 2 diabetics and 160 age and weight matched non-diabetic controls. They also found that leptin levels were lower in the diabetic population. In his study on

leptin in subjects with type 2 diabetes in Sudan (n=104), the main novel finding of Abdelgadir, (2002) was that serum leptin was significantly lower in diabetic subjects compared with controls in both females (P = .0001) and males (P = .019). Abu sayeed et al. (2003) reported that although leptin levels are increased in obesity, obese subjects with type 2 diabetes did not display high leptin levels. They examined whether leptin levels are also reduced in fifty lean Bangladeshi women with type 2 diabetes aged 37.2±1.3 years compared to 50 nondiabetic women aged 33.4±1.9 years served as control subjects. Diabetic subjects had lower leptin compared to controls (11.1±1.6 vs. 16.2±1.9 ng/ml, P=0.001).

On the other hand, higher serum leptin levels were reported in type 2 diabetic patients with diabetic nephropathy compared to controls (Verrotti et al., 2001 and El Meligi et al., 2003). Such heterogensity of leptin could be explained partially on the basis that patients in this studies differ from our study in being had diabetic nephropathy. This implies that with diabetes progress the kidney loose its function progressively and this will limit its ability to remove leptin from plasma (Meyer et al., 1997), therefore its level will increase in diabetic patients with diabetic nephropathy.

In the present study the t-test showed that the individuals who were not restricted to diet had significantly higher leptin levels than those who did. Such individuals tend to be obese. To test this idea we relate BMI with leptin levels of the study population. ANOVA test revealed positive association between BMI and leptin i.e. the larger the BMI, the higher the level of leptin. This means that leptin levels are higher in obese subjects. Our finding is in agreement with other authors who found significant positive correlation between serum leptin and BMI (Mohamed Ali et al., 1997, Nakazona et al, 1998, Fruehwald-Schultes et al., 1999, Nevalanen et al., 2000, Mohiti et al., 2005 and zabut et al, 2007). On the other hand, El Meligi et al., (2003) showed no correlation between leptin and BMI . They explained their result by the presence of an additional factor which is diabetic nephropathy.

Although leptin is a circulating signal that reduces appetite, in general, obese people have an unusually high circulating concentration of leptin.

(Considine, et al 1996). These people are resistant to the effects of insulin. Leptin could be regulated by insulin (Susan, 2000 and Mohiti et al., 2005). The high sustained concentrations of leptin form the enlarged adipose stores result in leptin desensitization. Disrupting leptin's appetite-controlling passageways leads to disturbance in two different brain body pathways: 1) pathway responsible for controlling appetite and fat storage which leads to increase eating and fat storage and 2) pathway responsible for telling the liver what to do with its stored glucose. Impairment of these two pathways leads to obesity, and obesity is known to significantly raise the risk of diabetes. However, it may take disruptions to both pathways to bring on full-blown diabetes and overwhelm the body's ability to control blood glucose levels via the action of insulin (American Diabetes Association, 2005).

As mentioned earlier our results showed higher levels of leptin in obese individuals. Since obesity is a major risk factor for diabetes, one can expect to find higher levels of leptin in our diabetic patients compared to controls. However, the result presented here contradict the expectation and showed lower leptin levels in diabetic patients. This result agreed with many studies (Liu et al., 1999, Tatti et al., 2001 and Abdelgadir, 2002). The contradiction could be explained on the basis of: 1) Many of the control females are obese, 2) More than half of our patients were diabetics since less than 5 years i.e few of them developed microalbuminurea in this short period. However, less than quarter of patients developed microalbuminurea probably in the later periods. This means that kidney is still efficiently remove leptin from the plasma.

On the light of the previous results, one can say that obese individuals had higher levels of serum leptin and since obesity is a major risk factor for diabetes, leptin level will be raised in diabetic patients but probably as the disease progress i.e in the stage of diabetic nephropathy, not in early stages of the disease as we noted in our study. It was suggested that leptin levels may increase with the progression of diabetic nephropathy (Fruchwald-Schultes et al., 1999 and El Meligi et al., 2003).

In this study it was found a significant negative correlation between leptin and TGL or HDL-C. Such correlations coincide with the above mentioned findings

that diabetic patients showed lower leptin levels, elevated triglyceride levels and lower HDL-C levels compared to controls. Similar results were obtained (Nevalinen et al., 2000 and Mohiti et a., 2005). However, Nakazona et al. (1998) and El Meligi et al. (2003) reported positive correlation between leptin and triglyceride levels and no correlation between leptin and HDL-C levels in diabetic patients with diabetic nephropathy and patients on hemodialysis.

Diabetic patients showed higher albumin levels in their urine compared to controls. However, there is a large amount of variation in urinary albumin for both controls and patients. Such variation is common in females due to excess epithelial tissues in urine and renders the statistical power of significance to be reduced. Microalbuminuric controls and patients were 7 (9.5%) and 20 (24.7%), repectively (χ^2 =3.59, p=0.045). This means that the majority of the patients did not display kidney function impairment which coincide with unchangeable creatnine levels both in serum and urine i.e. the kidney is still efficient to remove leptin hormone from patients' blood as discussed earlier.

This finding on microalbuminurea is in agreement with previous study carried out in the Gaza Strip (Altibi and Yassin, 2008) who demonstrated that 22.2% of the study population were microalbuminuric. In addition, the previous study showed that 22.2% of the study population were macroalbuminuric. In other countries including Saudi Arabia and China the prevalence of microalbuminuria was higher than that found in our study with percentages of 49.3 and 41.4%, respectively (Al Ghamdi, 2000 and Lu et al., 2007) . However, the same studies showed that macroalbuminuria were prevalent with percentages of 5.5 and 8.2%, respectively. Such data means that the prevalence of microalbuminuria varied among different countries. However, the high prevalence of microalbuminuria in the previous mentioned countries than in the GS could be attributed to poor following of the American Diabetes Association recommendations by these countries for reducing and slowing the progression of nephropathy by optimizing control of glucose and blood pressure (American Diabetes Association, 2002). In the context of such comparison and as discussed earlier, most of diabetic patients, particularly the

well educated ones of this study population had a better control of the disease through adoption of healthy diets.

On the light of this results, it is acceptable to state that "although leptin levels are increased in obesity, obese subjects with type 2 diabetes are not necessarily displaing high leptin levels". This could be applied on the early stages of the disease. However, elevation of leptin levels were commonly noticed with progress of diabetes where kidney function for leptin removal is usually affected.

Chapter 6

Conclusions and Recommendations

6.1 Conclusions

1. Diabetes was found to be associated with family history, diet and obesity among the females.

2. The main self-reported complications among patients were retinopathy, neuropathy and cardiovascular diseases. The longer the duration of diabetes, the higher the percentage of self reported complications.

3. Cholesterol, triglycerides and low density lipoprotein cholesterol were significantly higher in diabetics, whereas high density lipoprotein cholesterol was significantly lower

4. Serum leptin levels were significantly lower in diabetic patients compared to controls.

5. Leptin was negatively correlated with blood glucose and triglyceride levels whereas it correlates positively with body mass index and HDL-C level

6. Individuals who were not restricted to diet had higher leptin levels than those who did

7. Diabetic patients showed higher albumin levels in their urine. Creatinine level was normal in does blood and urine. Albumin/creatinine ratio was increased in patients. Microalbuminuria was higher among patients.

6.2 Recommendations

1. Control of obesity by limiting fat intake, increasing consumption of fruit and vegetables, as well as legumes, whole grains and nuts; limiting sugar intake and increasing physical activity.

2. Follow healthy diet regime and eating healthy food

3. Regular investigation of the complications related to diabetes

4. Enhancement of people awareness towards the disease by launching educational programs and workshops on diabetes.

5. Testing of leptin hormone and lipid profile particularly in the early stages of diabetes

6. Frequent monitoring of microalbuminurea and albumin/creatinine ratio to avoid the future development of diabetic nephropathy.

7. Further studies are needed to clarify the role of leptin as "a missing link" between obesity and diabetes which may be useful in controlling and prevention of the disease.

Chapter 7

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Annex 1

Palestinian National Authority
Ministry of Health
Helsinki Committee

السلطة الوطنية الفلسطينية
وزارة الصحة
لجنة هلسنكي

التاريخ2009/6/3

Name:

الاسم: هنان جمعة الطويل

I would like to inform you that the committee has discussed your application about:

يسرنا علماً بأن اللجنة قد ناقشت مقترح دراستكم حول:-

Leptin status and some Biochemical parameters among type2 diabetic Females in Gaza Governorate, Gaza Strip

In its meeting on June 2009

and decided the Following:-

To approve the above mention research study.

وذلك في جلستها المنعقدة لشهر 6 2009

و قد قررت ما يلي:-

الموافقة على البحث المذكور عاليه.

Signature
توقيع

Member
عضو

Member
عضو

Conditions:-

- Valid for 2 years from the date of approval to start.
- It is necessary to notify the committee in any change in the admitted study protocol.
- The committee appreciate receiving one copy of your final research when it is completed.

Annex 2

الجـــــــــامعة الإسلامية – غزة
The Islamic University of Gaza
كلية العلوم
مدير برنامج ماجستير العلوم الحياتية
التاريخ/ 2008/7/2م

الأخ الدكتور/ محمد الكاشف مدير عام المستشفيات حفظه الله ،،،

السلام عليكم ورحمة الله وبركاته .

الموضوع / تسهيل مهمة باحثة

نود أن نعلم سيادتكم بان الطالبـــة/ **حنـــان جمعـــة الطويـــل** طالبـــة فـــي ماجـــستير العلـــــــوم الحياتية في الجامعة الإسلامية تقوم بـــإجراء دراســـتها للماجـــستير.

وترغب في دخول مختبر الكيمياء وعيادة السكر في عيادة الرمال.

لذا نرجو من سيادتكم تسهيل مهمة الطالبة في الحصول علـــى المعلومـــات اللازمـــة لبحثها.

ولكم منا جزيل الشكر والتقدير ،،،

مدير برنامج ماجستير العلوم الحياتية
د. عبود يامر القشاوي

Islamic University P. Box 108 Al Rimal Gaza Palestine الجامعة الإسلامية غزة – الرمال ص.ب 108 فلسطين
Tel:(970/8)2860700 Fax:(970/8)2860700 2863552 e-mail:public@mail.iugaza.edu Web Site:www.iugaza.edu

Annex 3

الجـــــــامعة الإسلامية – غزة
The Islamic University of Gaza
مدير برنامج ماجستير العلوم الحياتية

كلية العلوم

التاريخ/ 28-1-2009م

الأخت الدكتورة/ رندة الخضري مدير عام المختبرات بوزارة الصحة حفظها الله ،،،

السلام عليكم ورحمة الله وبركاته

الموضوع / مساعدة الباحثة: حنان جمعة الطويل

نحيطكم علماً بأن الطالبة/ ***حنان جمعة الطويل*** والتي تحمل الرقم الجـــامعي ***22006-0144*** هي طالبة في برنامج ماجستير العلوم الحياتية بالجامعة الإسلامية وترغب في استخدام أجهزة مختبرات عيادة الرمال وذلك لإجراء عمليتي التخزين والتحليل.

لذا نرجو من سيادتكم مساعدة الباحثة في العمل.

ولكم منا جزيل الشكر والتقدير،،،

السيد/ مدير المختبرات المركزية المحترم

السيد/ مدير مختبر مركز الرمال المحترم

لاتاخ حسب النظام

مدير برنامج ماجستير العلوم الحياتية

د. عبود القيشاوي

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Tel:(970/8)2860700 Fax:(970/8)2860700 2863552 e-mail:public@mail.iugaza.edu Web Site:www.iugaza.edu

الجامعة الإسلامية-غزة – الرمال ص.ب:108 فلسطين

Annex 4

Control questionnaire

اخي المواطن الكريم/ ارجو مساعدتنا في اتمام هذه الدراسة (بحث ماجستير احياء / الجامعة الاسلامية) و التي تختص بمرضى السكري حيث ان هدفها منع او تاخير دخول المريض في الفشل الكلوي اهم مضاعفات المرض الخطيرة ، و ذلك من خلال تعبئة هذا الاستبيان وتبرعك لنا بعينة دم و عينة بول لاجراء بعض الفحوصات و التجارب عليها.

Personal data	
Name	
Age	
Education	Illiterate Primary Preparatory Secondary University or Diploma
Family history of diabetes	◯ Yes ◯ No
Obese	Weight: Kg Height: cm
Diet	◯ Yes ◯ No
Clinical data	
Retinopathy	◯ Yes ◯ No
Cardiovascular diseases	◯ Yes ◯ No
Neuropathy	◯ Yes ◯ No
Recurrent infections	◯ Yes ◯ No
Dermatopathy	◯ Yes ◯ No
Oral cavity lesions	◯ Yes ◯ No

Agreement: I agree to complete this questionnaire concerning my health statement.

انا موافق على تعبئة هذا الاستبيان الذي يتعلق بصحتي.

التوقيع:----------

التاريخ:---------- شكرا لكم على حسن تعاونكم

الباحثة / حنان الطويل

Annex 5

Patient questionnaire

اخي المواطن الكريم/ ارجو مساعدتنا في اتمام هذه الدراسة (بحث ماجستير احياء / الجامعة الاسلامية) - و التي تختص بمرضى السكري حيث ان هدفها منع او تاخير دخول المريض في الفشل الكلوي اهم مضاعفات المـــرض الخطيرة، و ذلك من خلال تعبئة هذا الاستبيان وتبرعك لنا بعينة دم و عينة بول لاجراء بعـــض الفحوصـــات و التجارب عليها.

Personal data	
Name	
Age	
Age at diagnosis	
Education	Illiterate Primary Preparatory Secondary University or Diploma
Family history of diabetes	◯ Yes ◯ No
Obese	Weight: Kg Height: cm
Diet	◯ Yes ◯ No
Clinical data	
Duration of DM	
Complications	
Retinopathy	◯ Yes ◯ No
Cardiovascular diseases	◯ Yes ◯ No
Neuropathy	◯ Yes ◯ No
Recurrent infections	◯ Yes ◯ No
Dermatopathy	◯ Yes ◯ No
Oral cavity lesions	◯ Yes ◯ No

Agreement: I agree to complete this questionnaire concerning my health statement.

انا موافق على تعبئة هذا الاستبيان الذي يتعلق بصحتي.

التوقيع:----------

التاريخ:---------- شكرا لكم على حسن تعاونكم

الباحثة / حنان الطويل