Serum Vaspin Level in Type 2 Diabetic Patients from Gaza Strip

مستوى الفازبين في مصل الدم لمرضى السكرى من النوع الثاني في قطاع غزة

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**A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of
Master of Biological Science/Medical Technology**

November/2016

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إقــــــرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

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نتيجة الحكم على أطروحة ماجستير

بنــاءً على موافقــة شــئون البحــث العلمــي والدراســات العليــا بالجامعــة الإسلامية بغـزة علــى تشـكيل لجنــة الحكم على أطروحـة الباحثـة/ **نجـوى طـلال خمـيس البورنـو** لنيل درجـة الماجستير فـي كليـة *العلـوم* قسم العلوم الحياتية– تحاليل طبية وموضوعها:

مستوى الفازبين في مصل الدم لمرضى السكري من النوع الثاني في قطاع غزة
Serum Vaspin Level in Type 2 Diabetic Patients from Gaza strip

وبعد المناقشـة التـي تمـت اليـوم السبت 12 صفـر 1438هـ، الموافـق 2016/11/12 السـاعة الواحدة ظهراً في قاعة مؤتمرات مبنى اللحيدان، اجتمعت لجنة الحكم على الأطروحة والمكونة من:

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د. محمـود اسمـاعيل الحبيبـي	مناقشـاً خارجيـاً	....

وبعد المداولة أوصت اللجنة بمنح الباحث درجة الماجستير في كلية *العلوم*/ قسم العلوم الحياتية – تحاليل طبية.

واللجنـة إذ تمنحـه هذه الدرجة فإنها توصيه بتقوى الله ولزوم طاعته وأن يسخر علمه في خدمة دينه ووطنه.

والله ولي التوفيق ،،،

نائب الرئيس لشئون البحث العلمي والدراسات العليا

أ.د. عبدالرؤوف علي المناعمة

Abstract

Background: Type 2 diabetes is prevalent in Gaza strip. Recently, vaspin has been identified as interesting novel adipokine having insulin sensitizing effect. However, the relationship between vaspin and type 2diabetes have not been adequately studied.

Objective: To assess serum vaspin level in type 2 diabetic patients from Gaza Strip, and its relationships with some biochemical variable.

Materials and methods: This case-control study comprised 88 type 2 diabetic patients (44 males and 44 females) and 88 healthy controls (44 males and 44 females). Questionnaire interview was applied. Serum vaspin, glucose, blood glycated hemoglobin (HbA1c), insulin, cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine and Homeostasis Model Assessment Insulin Resistance (HOMA-IR) were determined. Data were computer analyzed using SPSS version 22.0.

Results: The mean ages of cases and controls were 45.9±7.1 and 45.8±7.0 years, respectively. The mean level of vaspin was significantly higher in cases compared to controls (0.73±0.67 *versus* 0.47±0.43 ng/ml, P=0.033). Obese diabetic patients showed higher vaspin levels compared to controls (0.99±0.7 *versus* 0.62±0.52 ng/ml, P=0.048). Vaspin was also significantly increased in obese cases compared to non obese cases (0.99±0.7 *versus* 0.48±0.42 ng/ml, P=0.005). The levels of blood HbA1c, serum glucose and HOMA-IR were significantly increased in cases compared to controls (6.7±1.3 *versus* 4.7±0.65%, P=0.000 and 189.6±79.1 *versus* 90.7±13.6 mg/dl, P=0.000, 8.0±7.3 *versus* 3.6±2.4, P=0.000, respectively). The mean levels of triglycerides was significantly higher in cases compared to controls (187.6±133.8 *versus* 133.7±71.8 mg/dl, P=0.021) whereas HDL-C was significantly lower in cases (45.3±10.3 *versus* 50.8±13.8 mg/dl, P=0.039). Serum vaspin levels showed significant positive correlations with BMI (r=0.280, P=0.008), HbA1c% (r=0.209, P=0.049), glucose (r=0.290, P =0.006), HOMA-IR (r=0.276, P=0.010), triglycerides (r=0.303, P=0.004) and ALT activity (r=0.434, P=0.000).

Conclusions: Serum vaspin was significantly higher in type 2 diabetic patients compared to controls. Serum vaspin levels showed significant positive correlations with BMI, HbA1c, glucose, HOMA-IR, triglycerides and ALT.

Keywords: Type 2 diabetic patients, Serum vaspin, Gaza strip.

ملخص الدراسة

المقدمة: السكري من النوع الثاني هو الأكثر شيوعا في قطاع غزة. مؤخرا تم التعرف على الفازبين كنوع جديد من اديبوكاين، لديه القدرة على التحسس للأنسولين ومع ذلك فان العلاقة بين الفازبين و داء السكري لم تدرس بشكل كاف.

الهدف: تقييم مستوى الفازبين لدى مرضى السكري من النوع الثاني في قطاع غزة , وعلاقته ببعض المتغيرات البيوكيميائية.

الطرق والأدوات: منهج الدراسة (مجموعة مرضية – مجموعة ضابطة) المجموعة المرضية تحتوي على88 مريض سكر(44 رجل -44 امرأة) 88 شخصا من الأصحاء (44 رجل -44 امرأة). و قد تم إجراء المقابلة الشخصية لتعبئة الاستبيان، وتم قياس مستوى الفازبين، الجلوكوز، مخزون السكر، الأنسولين، الكولسترول، ألدهون الثلاثية، البروتين ألدهني عالي الكثافة، البروتين ألدهني منخفض الكثافة، الإنزيم الناقل للألانين أمين، الإنزيم الناقل للأسبارتات أمين، اليوريا، الكرياتينين و مقاومة الأنسولين. وتم تحليل البيانات والنتائج باستخدام البرنامج الإحصائي SPSS-22 .

النتائج: كان متوسط الأعمار في الحالات والضوابط هو45.9±7.1 و45.8±7.0 سنة على التوالي. كان متوسط مستوى الفازبين في الحالات أكثر بكثير مقارنة مع الضوابط وهذه كانت ذات دلالة إحصائية (0.73±0.67 للمرضى مقابل 0.47±0.43 نانوجرام/مل للاصحاء, P=0.033)، وكما وجد أن مستوى الفازبين لدى المرضى البدناء أكثر من الضوابط (0.7±0.99 للمرضى مقابل 0.52±0.62 نانوجرام/مل للاصحاء، P=0.005). وكما وجد أيضا أن مستوى الفازبين زاد بشكل ملحوظ بين المرضى البد ناء مقارنة مع المرضى الضعفاء (0.7±0.99 للمرضى مقابل 0.48±0.42 نانوجرام/مل للاصحاء ، P=0.005) وزادت مستويات نسبة مخزون السكر، الجلوكوز ومقاومة الأنسولين في الدم بشكل ملحوظ في الحالات بالمقارنة مع الضوابط.(6.7±1.3 للمرضى مقابل 4.7±0.65%، 189.6±79.1 للمرضى مقابل 90.7±13.6 للاصحاء ملجرام/100ملل، 8.0±7.3 للمرضى مقابل 3.6±2.4 % للمرضى، P=0.000، على التوالى) وكانت المستويات الدهون الثلاثية أعلى بكثير في الحالات مقارنة مع الضوابط.(133.8±187.6 للمرضى مقابل 71.8±133.7 ملجرام/100ملل، P=0.021)، في حين أن ا لبروتين الدهني عالي الكثافة كان أقل من ذلك بكثير في الحالات مقارنة بالضوابط وكانت هذه النتيجة ذات دلالة إحصائية (45.3±10.3 مقابل 50.8±13.8 ملجرام/100ملل، P=0.039)، كما أظهرت الدراسة أن الفازبين كان له ارتباط ايجابي مع كل كتلة الجسم(r=0.280, P=0.008)، مخزون السكر(r=0.209, P=0.049)، الجلوكوز,(r=0.290, P =0.006) مقاومة الأنسولين(r=0.276, P=0.010)، الدهون الثلاثية (r=0.303, P=0.004) والإنزيم الناقل للألانين (r=0.434, P=0.000).

الاستنتاج: يوجد ارتفاع في مستوى الفازبين لدي مرضى السكر من النوع الثاني، ولوحظ أن هناك علاقة ايجابية بين مستويات الفازبين وكتلة الجسم، مخزون السكر، السكر، مقاومة الأنسولين، الدهون الثلاثية و الإنزيم الناقل للألانين أمين .

الكلمات المفتاحية : سكري النمط الثاني، الفازبين، قطاع غزة

III

Dedication

I dedicate this work to:

My beloved mother who has always supporting me

To my father spirit

To martyrs Mohamed and Mahmoud Al Borno

To my sister and brother

All researchers who are working to improve the quality of life

Dedication is almost expressed to the Palestinian people who have suffered and will

be struggling with the persistence to have a free Palestine.

Najwa T. Al Borno

Acknowledgment

I would like to express my deepest gratitude and appreciation to my supervisor **Prof. Dr Maged M. Yassin,** Professor of Physiology, Faculty of Medicine, The Islamic University of Gaza for his planning and initiating of this work and for his continuous support, encouragement and kind of supervision that leads to the emergence of this work in its current form.

Special thanks go to **Dr. Kamal Elkhalout,** Assistant Professor of Biotechnology, Faculty of Science, The Islamic University of Gaza for his support and valuable discussion throughout reading of thesis and for his scientific advices.

Special thanks for the dearest persons to me my **mother**, my brothers and my sisters for their support and encouragements.

I would like to thank the staff of Diabetic Units in primary health care for their facilitation and helping me in samples collection and sample separation.

Special thanks to Palestinian Medical Relief Society for helping me in vaspin analysis.

My special thanks to **Mr. Abed EL- Rahman Hamad** for his help in statistical analysis.

At the end, I am very grateful to every person who participated and helped me to complete this study.

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List of Abbreviations

VASPIN   Visceral Adipose Serine Protease Inhibitors

NGT   Normal Glucose Tolerance

HOMA-IR   Homeostasis Model Assessment Insulin Resistance

BMI   Body Mass Index.

HDL-C   High Density Lipoprotein.

LDL-C   Low Density Lipoprotein.

AST   Aspartate aminotransferase (AST).

ALT   Alanine aminotransferase (ALT).

hk7   human kallikrein 7.

SNPs   Single nucleotide polymorphisms

OLETF   Otsuka Long-Evans Tokushima Fatty rats

Chapter 1
Introduction

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Introduction

1.1 Overview

Diabetes mellitus is a chronic metabolic disorder characterized by presence of chronic hyperglycemia accompanied by greater or lesser impairment in the metabolism of carbohydrates, lipids and proteins. The origin and etiology of diabetes mellitus can vary greatly but always include defects in either insulin secretion or response or in both at some point in the course of the disease (Conget, 2002).

There are two major types of diabetes mellitus: Type 1 diabetes which is primarily a result of pancreatic β-cell destruction due to an immune-mediated process that is likely incited by environmental factors in genetically predisposed individuals (Harjutsalo, Reunanen & Tuomilehto, 2006). 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 development of overt hyperglycemia, especially fasting hyperglycemia (Michael, 2000; Guyton & Hall, 2006 and Holt & Hanley, 2012). In such conditions, lipolysis in adipose tissue is promoted leading to elevated circulating levels of free fatty acids. 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, Sarkadi-Nagy, Duncan, Ahmadian & Sul, 2007). In addition, disturbance in liver and kidney functions was also reported in type 2 diabetes (Sharma, Hirulkar, Wadel & Das, 2011; Yassin, Altibi & El shanti 2011 and Atiba et al., 2013).

Vaspin, a member of the serine protease inhibitor family, is an adipocytokine that has been isolated from the visceral adipose tissue of Otsuka Long-Evans Tokushima Fatty (OLETF) rats (Lago, Dieguez, Gomerreino & Gualillo, 2007). In humans, vaspin expression has been observed in the adipose tissue, (Klöting et al.,

2006) stomach, (Klöting et al., 2011) liver, and pancreas (Körner et al., 2010). Evidence in obese and diabetic Otsuka Long-Evans Tokushima rats (OLEFT), suggests that vaspin improves insulin action and glucose tolerance. Its levels decreased significantly with the worsening of diabetes (Hida et al., 2005). Vaspin might be involved in the pathogenesis and progression of cardiovascular disease since it is also expressed in periadventitial and epicardial adipose tissue as well as in vascular smooth cells (Spiroglou, Kostopoulos, Varakis & Papadaki, 2010)

1.2   Significance

In Gaza strip, no previous study linked vaspin to diabetes mellitus. Therefore, the present study is the first to assess serum vaspin level in type 2 diabetic patients from Gaza strip in relation to some of biochemical indices.

1.3   General objective

To asses serum vaspin level in type 2 diabetic patients from Gaza strip.

1.4   Specific objectives

1. To determine serum vaspin level in cases compared to controls.
2. To estimate whole blood HbA1c, serum glucose and insulin.
3. To measure lipid profile including cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) in cases compared to controls.
4. To test liver function throughout measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST).
5. To examine kidney function throughout estimation of urea and creatinine in cases and controls..
6. To verify the relationship between vaspin and the studied parameters of the study population

Chapter 2
Literature Review

Chapter 2

Literature Review

2.1 Definition of diabetes mellitus

Diabetes mellitus is a chronic disease that affects the lives of millions around the world (International Diabetes Federation, IDF, 2006). Diabetes mellitus is defined as diabetes treated by diet alone or by diet combined with oral hypoglycemic agents or as treatment with insulin (Tanriverd, 2011). It is a metabolic disorder characterized by chronic hyperglycemia due to disturbances of carbohydrate, fat and protein metabolism that are associated with absolute or relative deficiencies in insulin secretion, insulin action or both. Diabetes mellitus possess a major and growing health and socioeconomic burden on society that affects over 177 million people worldwide and this figure is likely to be more than double by the year 2030 (World Health Organization, WHO, 2003).

2.2 Types of diabetes

The most common types of diabetes mellitus are:

2.2.1 Type 1 diabetes

This form of diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin dependent diabetes, type 1, or juvenile-onset diabetes, results from a cellular mediated autoimmune destruction of the β-cells of the pancreas. In this form of diabetes the rate of β-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults) (American Diabetes Association, ADA, 2012).

2.2.2 Type 2 diabetes

Type 2 diabetes accounts for about 90-95% of all diagnosed cases of diabetes. Type 2 diabetes is characterized by insulin resistance and ongoing decline in β-cell function, glucose levels likely will worsen over time (Turner, Cull & Frighi, 1999), and treatment must be dynamic as therapeutic requirements increase with longer duration of disease. Type 2 diabetes develops in individuals who fail to compensate for insulin resistance by increasing pancreatic insulin secretion. Then, insulin deficiency results from pancreatic β-cell dysfunction and death (Cnop, 2008).

2.2.3 Gestational diabetes

Gestational diabetes has been defined as any degree of glucose intolerance with onset or first recognition during pregnancy. Although most cases resolve with delivery, the definition applied whether or not the condition persisted after pregnancy (ADA, 2012). The risk for developing type 2 diabetes within the first decade following pregnancy in gestational diabetes cases ranges between 35% and 60% (Seniuk, Ozegowska & Szczapa, 2009). Similarly, children of women with gestational diabetes are known to be at risk for obesity and diabetes mellitus in their later life (Bánhidy, Acs, Puho & Czeizel, 2010).

2.3 Type 2 diabetes

2.3.1 Metabolism in type 2 diabetes

Circulating glucose is derived from 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 hepatic processes including glycogenolysis and gluconeogenesis (Stephen, Aronoff, Kathy Berkowitz, Shreiner & Laura Want, 2004). 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 (Mather and Pollock, 2011).

The rate of glucose entering the circulation balanced by the rate of glucose removal from the circulation. 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. Both insulin and amylin are derived from the β-cells, glucagon from the α-cells of the pancreas, and GLP-1 and GIP from the L-cells of the intestine (ADA, 2004 and Wachters-Hagedoorn et al., 2006).

In the bi-hormonal model of glucose homeostasis, insulin is the key regulatory hormone of glucose disappearance, and glucagon is a major regulator of

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glucose appearance. After reaching a post-meal peak, blood glucose slowly decreases during the next several hours, eventually returning to fasting levels (Shrayyef & Gerich, 2010). 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 the direct action of insulin on the liver, and the paracrine effect or direct communication within the pancreas between the α- and β-cells, which results in glucagon suppression (Camacho, Pencek, Lacy, James & Wasserman, 2004).

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 development of overt hyperglycemia, especially fasting hyperglycemia (Michael, 2000; Guyton & Hall, 2006 and Holt & Hanley, 2012).

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. 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 (Bitzur, Cohen, Kamari, Shaish & Harates, 2009). In contrast, other studies documented that HDL-C was decreased (Yassin et al., 2011). In addition, disturbance in serum urea and creatinine was also reported in type 2 diabetes (Sharma et al., 2011).

2.3.2 Prevalence and mortality rate of type 2 diabetes mellitus

The world prevalence of diabetes in 2010 among adults aged 20-79 years is estimated to 6.4%, affecting 285 million adults. Between 2010 and 2030, there is an expected 70% increase in numbers of adults with diabetes in developing countries and a 20% increase in developed countries (Shaw, Sicree & Zimmet, 2010). Each year more than 231,000 people in the United States and more than 3,96 million

people worldwide die from diabetes and its complications (IDF, 2009). The prevalence rate of diabetes mellitus in Palestine is about 9% in 2000 (Ministry of Health, 2002). This study was conducted in 2000 in cooperation with Al-Quds university and MOH. It is around the reported prevalence rate in Egypt and Tunisia (9%) and less than Saudi Arabia (12%) and Oman (13%). By the end of 2003, routine data gathered by the United Nation Relief and Works Agency showed that the prevalence rate was 10.5% in the West Bank and 11.8% in the Gaza Strip among the registered Palestinian refugees aged 40 years and older. The rate of reported diabetes mellitus was 7.2% at age 40–49 years, 19.1% at 50–59 years, and 24.8% at 60 years and older (Palestinian Central Bureau of Statistics, PCBS, 2006). In 2011, the total number of new reported cases of diabetes mellitus in West Bank was 3984 with incidence rate 154.4 per 100,000 of population (MOH, 2012). In 2015, the total number of new reported cases of diabetes mellitus in West Bank was 5,761 with incidence rate of 201.3 per 100,000 of population (MOH, 2015). In 2015, the total number of reported deaths in Palestine was 12,690, out of them 6,676 males and formed 52.6% and 6,014 were females and formed 47.4%. Also 7,714 out of total number of reported deaths were in West Bank, and out of them 4,139 were males and 3,575 were females, 4,976 deaths were reported in Gaza Strip, out of them 2,537were males and 2,439 were females (MOH, 2015).

2.3.3 Risk factors of type 2 diabetes

2.3.3.1 Body mass index

Many studies have reported that increased body mass index (BMI) is a strong risk factor for type 2 diabetes (Almdal, Scharling, Jensen & Vestergaard, 2008 and ADA, 2013). A strong positive association between obesity and type 2 diabetes was found both in men and women (Almdal et al., 2008 and Eckel et al., 2011). Obesity is associated with increased risk of developing insulin resistance and type 2 diabetes. In obese individuals adipose tissue releases increased amounts of non esterified fatty acids, glycerol, hormones, pro-inflammatory cytokines and other factors involved in the development of insulin resistance (Kahn, Hull & Utzschneider, 2006 and Ebe , Adamo & Caprio, 2011). When insulin resistance is accompanied by dysfunction of the β-cells, the following fall in insulin secretion results in failure to control blood glucose level leading to type 2 diabetes (Hebebrand & Hinney, 2009).

2.3.3.2 Lipids

Unfavorable blood lipids has been reported as a risk factor for type 2 diabetes (Almdal et al., 2008 and Rutter & nesto, 2011). An inverse relationship between HDL-C and risk of type 2 diabetes have been documented (Jacobsen, Bønaa & Njølstd, 2002 and Yassin et al., 2011). High plasma triglycerides and low plasma HDL-C levels are both seen in the insulin resistance syndrome (Taskinen, 2003; Bitzur et al., 2009 and Salazar et al., 2013).

2.3.3.3 Physical inactivity

Recent studies have found that physical inactivity is a risk factor for type 2 diabetes (Fretts et al., 2009; Colberg, 2012 and Steinbrecher et al., 2012). Prolonged television watching as a surrogate marker of sedentary lifestyle, was reported to be positively associated with diabetes risk in both men and women (Hu, Li, Colditz, Willett & Manson, 2003 and Krishnan, Rosenberg & Palmer, 2008). Moderate and vigorous physical activity was associated with a lower risk of type 2 diabetes (Weinstein et al., 2004 and Fretts et al., 2009). Physical activity plays an important role in delaying or prevent of development of type 2 diabetes in those at risk both directly by improving insulin sensitivity and reducing insulin resistance, and indirectly by beneficial changes in body mass and body composition (Kay & Singh, 2006 and ADA, 2013).

2.3.3.4 Dietary pattern

An important life style factor associated with the development of type 2 diabetes is dietary habits. Positive association have been reported between the risk of type 2 diabetes and different patterns of food intake (Liese, Weis, Schulz & Tooze, 2008 and Kurotani et al., 2012). Higher dietary glycemic index has been consistently associated with elevated risk of type 2 diabetes (Villegas et al., 2011 and Salvado, Gonaz'lez, Bullo & Ros, 2011). A review which included 19 studies, "On diet and risk of type 2 diabetes: the role of fat and carbohydrate" concluded that a higher intake of polyunsaturated fat and long- chain fatty acid is beneficial, where as higher intake of saturated fat and trans fat adversely affects glucose metabolism and insulin resistance (Hu, Liu & Dam, 2001). Another study found, higher consumption of butter, potatoes and whole milk to be associated with increased risk of type 2 diabetes. Higher consumption of fruits and vegetable was associated with reduced

risk of type 2 diabetes (Montonen, 2005). The possible mechanisms suggested are that insoluble fibers intake was consistently associated with improved insulin sensitivity and decreases risk of type 2 diabetes (Meyer et al., 2000 and Robert, Arch, Mainous, Dana & Kit, 2012).

2.3.3.5 Genetics

Several studies have found that genetic components plays an important role in pathogenesis of type 2 diabetes. They reported that positive family history among first degree relatives confers an increased risk of type 2 diabetes and the risk is greater when both parents are affected (Amini & Janghorban 2007; Ma et al., 2008, Frank & Hu, 2011 and Omar, 2013). Data supported that, genetic factors predispose to development of type 2 diabetes by reducing insulin sensitivity and insulin secretion which deteriorate in parallel in most human type 2 diabetes cases (Das & Elbein, 2006). Recent studies have identified variants in 11 genes to be significantly associated with the risk of type 2 diabetes independently of other clinical risk factors and variants in 8 of these genes were associated with impaired beta-cell function (Bao et al., 2013 and Lyssenko & Laakso, 2013).

2.3.4 Complications of type 2 diabetes mellitus

Complications of type 2 diabetes include acute and chronic complications. The acute complications comprise diabetic ketoacidosis, hyperosmolar hyperglycemic satate, lactic acidosis and hypoglycemia. The chronic complications include diabetic retinopathy, diabetic neuropathy, diabetic nephropathy and cardiovascular disease (Susztak et al., 2003; Becker, 2009 and Yassin et al., 2011).

2.3.4.1 Diabetic retinopathy

Diabetic retinopathy may be the most common microvascular complication of diabetes. The risk of developing diabetic retinopathy or other microvascular complications of diabetes depends on both the duration and the severity of hyperglycemia. Retinopathy may begin to develop as early as 7 years before the diagnosis of diabetes in patients with type 2 diabetes. Retinopathy involves changes in the retina. 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. 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).

2.3.4.2 Cardiovascular disease

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 & Flyvbjerg, 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 (Savage, Petersen & Shulman, 2005).

2.3.4.3 Diabetic neuropathy

Diabetic neuropathy is recognized by the American Diabetes Association (ADA) as the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes (ADA, 2007). The risk of developing diabetic neuropathy is proportional to both the magnitude and duration of hyperglycemia, and some individuals may possess genetic attributes that affect their predisposition to developing such complications. 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

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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. 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 (Bansal, Kalita & Misra, 2006).

2.3.4.4 Diabetic nephropathy

Diabetic kidney disease, also known as diabetic nephropathy, is what happens when macrovascular complications and microvascular complications converge. The high blood pressure caused by macrovascular complications, combined with the small blood vessel damage caused by microvascular complications, together can cause kidney failure. Nephropathy is the leading cause of chronic renal failure worldwide and is responsible for renal failure in about one third of patients who undergo dialysis. One of the initial markers of this condition is microalbuminuria, which indicates an increased risk of progression to nephropathy as well as an elevated risk of cardiovascular events (Yadav, Tiwari & Dhanaraj, 2008 and Yassin et al., 2011)

2.4 Vaspin
2.4.1 Definition and Structure:

Vaspin (Visceral Adipose tissue-derived Serpin), a member of the serine protease inhibitor family, is an adipocytokine that has been isolated from the visceral adipose tissue of Otsuka Long-Evans Tokushima Fatty (OLETF) rats (Lago et al., 2007). It is likely that vaspin belongs to the serpins. They are a superfamily of proteins characterized by the presence of a core domain consisting of three β-sheets and nine α-helices. There are approximately 500 serpins and a phylogenetic analysis divides them into 16 classes and 10 highly diverged 'orphans'(Silverman et al., 2001 and Gettins, 2002). The gene that encodes for vaspin has a molecular size of 1.8 kbs (Hida et al., 2005), figure 2.1., and maps on the chromosome 14 (14q32.13). After

isolating and cloning vaspin molecule, it was revealed that vaspin cDNA consists of 1236, 1242 and 1245 nucleotides in rats, mice and humans, respectively. The corresponding proteins consist of 412, 414, and 415 aminoacids, respectively (Hida et al., 2005).
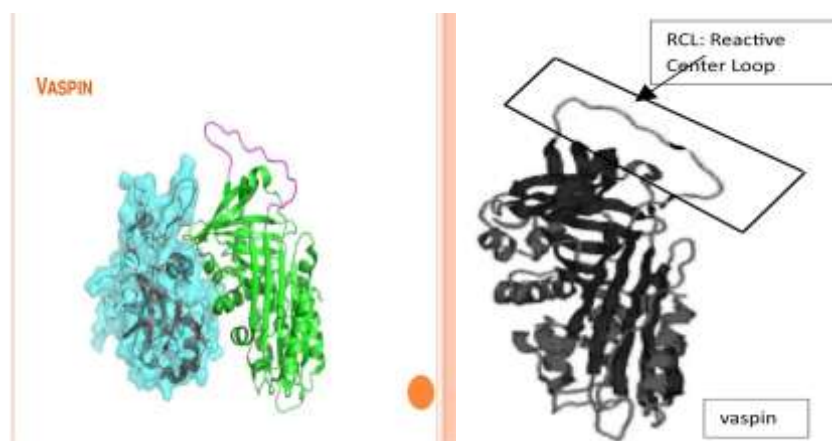
Figure (2.1): Molecular structure of vaspin (Hida et al ., 2005)

2.4.2 Sources of vaspin

In humans, vaspin expression has been observed in the adipose tissue (Klöting et al., 2006), stomach (Klöting et al., 2011), liver and pancreas (Körner et al., 2011). According to tissue expression pattern of vaspin mRNA in mouse and man, the expression level in almost tissue including white fat are rather low in lean human subject (BMI < 25 kg/m²) had undetectable vaspin mRNA in visceral and subcutaneous fat (Klöting et al., 2006).

2.4.3 Function of vaspin

Evidence in obese and diabetic Otsuka Long-Evans Tokushima rats (OLEFT), suggests that vaspin improves insulin action and glucose tolerance. Its levels decreased significantly with the worsening of diabetes (Hida et al., 2005). Vaspin has anti-inflammatory and anti-apoptotic effects on vascular cells as well as improving insulin resistance. Vaspin could inhibit inflammatory factor secretion from vascular smooth muscle cells and antagonize endothelial cell apoptosis induced by free fatty acid (Jung et al., 2011 and Phalitakul, Okada, Hara & Yamawaki 2011) .

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Data in humans is more controversial since some studies found vaspin expression as well as circulating levels of vaspin significantly increased in obese adults. Both concentrations increased in parallel with the raising of BMI and the worsening of glucose tolerance (Blüher, 2011; Youn et al., 2008 and Klöting et al., 2006). Vaspin might be involved in the pathogenesis and progression of cardiovascular disease since it is also expressed in periadventitial and epicardial adipose tissue as well as in vascular smooth cells (Spiroglosu et al., 2010).

2.4.4 Mechanism of action of Vaspin :

The mechanism of action of vaspin is not fully understood. The first protease, determined as a vaspin target, is human kallikrein7 (hK7), inhibited by classical serpin mechanism with high specificity in vitro. Heiker et al. (2013) have isolated vaspin-hK7 complexes in human plasma, established coexpression of both proteins in murine pancreatic β-cells, and exhibited the ability of hK7 to cleave human insulin within A- and B-chain. Treatment of isolated pancreatic islets with recombinant vaspin results in increased insulin concentration under conditions of glucose stimulation without affecting insulin secretion and a significant improvement in glucose tolerance in C57BL/6NTac and db/db mice, completely dependent on the serpin activity of vaspin and not related to vaspin-mediated changes in insulin sensitivity. hK7 inhibition seems to be the most likely underlying physiological mechanism for the compensatory vaspin effects on obesity-induced insulin resistance (Heiker et al., 2013).

2.4.5 Vaspin levels and biological actions in humans

The expression of vaspin mRNA in obese humans correlates with adipose tissue mass, while vaspin mRNA is not detectable in lean humans with normal glucose tolerance. These findings indicate that the expression of vaspin mRNA in the adipose tissue is regulated in a fat depot-specific manner and could represent a compensatory response associated with obesity, insulin resistance and type 2 diabetes mellitus (Klöting et at., 2006) . At present, there is no clear proof of a causal link between vaspin levels and visceral fat accumulation or insulin resistance (Li et al., 2008). However, it has been shown that vaspin levels are also gender-dependent, since women have significantly higher vaspin levels compared to men (Seeger et al.,

2008). Women with normal glucose tolerance have vaspin levels up to 2.5 times higher than men (Youn et al., 2008) . A recent study showed that serum vaspin levels present a meal-related diurnal variation (Jeong et al., 2010). Serum vaspin levels show a preprandial rise, 1-2 hours before the beginning of the meal. The higher levels were observed before lunch and the lowest before breakfast. Postprandially, vaspin levels decline gradually to preprandial levels within 2 hours after meal. Vaspin concentration also showed a nocturnal rise, with a peak at nighttime, when vaspin levels were approximately 250% higher than lowest daytime levels. This diurnal variation of serum vaspin concentration was exactly reciprocal to that of insulin and glucose variation. Insulin levels increase 4.7-8.3 times within thirty minutes after the beginning of the meal and gradually decline postprandially to reach preprandial levels before the next meal. In addition, insulin levels remain low during sleep (Jeong et al., 2010).

2.4.6 Vaspin and Type 2 diabetes

As the impact of vaspin gene variants is generally unknown, in MONICA/KORA F3 study, Kempf et al. (2010) aimed to explore the importance of SNPs (Single nucleotide polymorphisms) in the vaspin locus of chromosome 14 in the development of type 2 diabetes and obesity. Their results have shown a significant correlation between vaspin SNP rs2236242 and type 2 diabetes with genotype AA, carrying increased risk of glucose homeostasis disorders, and this association appears to be independent of BMI. Namely, there is a connection between vaspin and glucose metabolism, and vaspin could be regarded as a new link between obesity and related metabolic disorders, particularly diabetes (Kempf et al., 2010). Data on serum vaspin levels in type 2 diabetes are rather conflicting. Ye et al. (2009) have reported higher vaspin levels in subjects with type 2 diabetes and a positive correlation between vaspin and postprandial blood glucose levels. In addition Li et al. (2011) described a lowering effect of continuous subcutaneous insulin infusion on serum vaspin concentrations concomitantly with the beta-cell function amelioration in type 2 diabetes. Other studies have found no difference in vaspin levels between subjects with and without glucose abnormalities (Youn et al., 2008 and Feng et al., 2011), or recorded lower vaspin levels in the presence of type 2 diabetes (Breifeld et al., 2012 and Jian et al., 2014). Jian et al. (2014) have presumed

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that lower serum vaspin levels might serve as a risk factor for the development of type 2 diabetes (Jian et al., 2014). Elevated vaspin concentrations have been established in obese subjects with Normal Glucose Tolerance (NGT) and prediabetes, as well (Atya, Hassan, Amin & Ali, 2013). In addition, Atya et al. (2013) have reported a decline in vaspin levels with increasing the duration of diabetes. Li et al. (2011) have assessed vaspin in subjects with newly diagnosed type 2 diabetes, impaired glucose tolerance, and Normal Glucose Tolerance (NGT), following an intravenous 2-week insulin infusion. The circulating vaspin levels have been found significantly lower, and insulin sensitivity and glycemic control have been significantly improved in subjects with newly diagnosed type 2 diabetes. Changes in vaspin levels have positively correlated with the increase in insulin resistance, calculated indirectly by HOMA-IR. Based on the above data, it might be asserted that vaspin plays an important role in the pathogenesis of type 2 diabetes (El- Mesallamy, Kassem, El-Demererdash & Amin, 2011) .

The relationship between circulating vaspin levels and the presence of chronic complications of type 2 diabetes has also been assessed. Gulcelik et al. have demonstrated lower vaspin levels in females with type 2 diabetes and good glycemic control as compared to a group with poor glycemic control, and the presence of microvascular complications has been found to further reduce vaspin levels (Gulcelik, Karakaya, Gedik, Usman & Gurlek, 2008). Li et al. (2012) have examined vaspin levels in individuals with type 2 diabetes of up to 3-year duration, with or without macroangiopathy. They have found that the concentration of vaspin is raised in subjects with type 2 diabetes without carotid plaques as compared to Normal Glucose Tolerance (NGT) and diminished in subjects with type 2 diabetes with carotid plaques in comparison to those with type 2 diabetes without plaques. A significant negative correlation between the presence of carotid plaques and serum vaspin levels has been observed in subjects with type 2 diabetes of up to 3-year duration of the disease. The compensatory capacity of vaspin secretion gradually lessens with the increase in the duration of diabetes or the onset of cardiovascular diseases and aggravation of atherosclerosis, resulting in a slow decrease in vaspin levels, shown in several studies (Hida et al., 2005; Aust et al., 2009; Kadoglou et al., 2011 and Kobat et al., 2012)

Chapter 3
Materials and Methods

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3.1 Study design

The present study is a case control study.

3.2 Study population

The study population comprised type 2 diabetic patients aged 34-68 years attending Diabetic Units at Al-Remal Medical Center in Gaza strip, control group was apparently healthy normal persons.

3.3 Sample size and sampling

Non probability accidental sample of type 2 diabetic patients, previously diagnosed according to the World Health Organization diagnostic criteria for diabetes (WHO, 2006), were selected as cases from Diabetic Units at Al-Remal Medical Center in Gaza strip. Controls were apparently healthy non diabetic individuals selected from the general population. Cases and controls were age and gender matched. The sample size calculations were based on the formula for case-control studies. EPI-INFO statistical package version 3.5.1 was used with 95% CI, 80% power and 50% proportion as conservative and OR > 2. The sample size in case of 1:1 ratio of case control was found to be 88:88.

3.4 Exclusion criteria

- Cases and controls whose aged under 34 years and above 68 years old.
- Type 1 diabetic patients.
- Pregnant women.
- Patients who take hormone replacement therapy or corticosteroid therapy.

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3.5 Ethical consideration

An official letter of request sent from the Palestinian Ministry of Health to primary health care in Gaza Strip to facilitate the conduction of the study (Annex 1). Helsiniki committee for ethical approval were taken (Annex3). The participants were given a full explanation about the purpose of the study and assurance about the confidentiality of the information and the participation was optional.

3.6 Limitation of the study

Sample collection was relatively difficult as many patients refuse to participate in the study.

3.7 Data collection

3.7.1 Questionnaire interview

A meeting interview was used for filling in a questionnaire which designed for matching the study need for both cases and controls (Annex 2). The questionnaire included questions on the personal profile of the study population (Age, gender and education), socioeconomic data (employment, family income/month, family history of diabetes and smoking), physical activity, meal frequency/day, duration of diabetes and self-reported complications (retinopathy, cardiovascular disease and neuropathy) among the study population

3.7.2 Body mass index

Body mass index was calculated as the ratio of body weight in Kg/height in square meter. Patients were asked to remove heavy clothes and shoes before measurement of weight and height. Medical balance (Seca Model 762, Germany) was used for weight measurement. 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, 2012).

3.7.3 Collection and processing

Blood Sample were collected from patients and controls. Twelve hours fasting overnight venous blood samples were collected from 88 type 2 diabetic patients and 88 healthy controls. Blood samples (8 ml each) ware drawn into vacutainer from each control and diabetic patients. About 2 ml blood was placed into ethylene diamine tetra acetic acid (EDTA) vacutainer tube to perform HbA1c for cases and controls. The remainder quantity of blood (6 ml) was placed in plan tube and was left for a while without anticoagulant to allow blood to clot. Serum samples were obtained by centrifugation at 3000 rpm for 10 minutes for determination of vaspin, insulin, glucose, cholesterol, triglycerides, HDL-C, LDL-C, ALT, AST, urea and creatinine.

3.8 Biochemical analysis.

3.8.1 Determination of serum vaspin.

Serum vaspin was determined by enzyme linked immunoassay (ELISA) (Youn et al., 2008).

Principle of ELISA test .

The enzyme immunoassay for vaspin is so called sandwich assay. It utilizes specific and high affinity polyclonal antibodies for this protein the vaspin in samples bind to the immobilized first antibody on microtiter plate. In the following steps, the biotinylated antibody bind in turn to vaspin. After washing, streptavidin –peroxidase –enzyme conjugate will add, which will bind highly specific to the biotin and will catalyze the enzymatic reaction, which turns the colour of substrate, quantitatively depend on the vaspin level of sample. Table 3.1 illustrate composition of reagents for ELISA test.

Table (3.1): Composition of reagents for ELISA vaspin kit

Materials provided 96 Tests		
MTP	Microwell plate coated with anti human vaspin antibody12	12x8x1
STD	Standard A-E , lyophilized , contain recombinant vaspin .standard value between 0.01-1ng/ml vaspin and have to be reconstituted with 750μl(each) dilution buffer	0.75 ml
Control	Vaspin Control Sera KS1 and KS2 vials 250µl, lypholized contain human serum and shoud reconstituted in each 250 µl dilution buffer	0.25 ml
Ab	Antibody conjugate AK, ready for use	12 ml
BUF	Dilution buffer VP 1 bottle	120 ml
CONJ	Enzyme Conjugate EK, 12 ml, ready for use, contains horseradisch-peroxidase conjugate to streptavidin, Use 100 µl for each well in the assay.	12 Ml
SUBST	Substrate (S), 12 ml, ready for use, horseradish-peroxidase-(HRP)-substrate, stabilized H2O2 Tetramethylbencidine	12 ml
H_2SO_4	Solution (SL), 12 ml**,** ready for use, 0.2 M sulphuric acid, Caution acid!	12 ml
	Sealing tape for covering of the microtiter plate, 3 x, adhesive.	3

Preparation of reagents for ELISA test:

Before running the test, prepare the following:

Standards and control

For the reconstitution of the lyophilised Standards A - E Dilution Buffer VP has to be used. The lyophilised Control Sera KS1 and KS2 must be reconstituted with the Dilution Buffer VP. The dilution should be according to the dilution of the respected samples. It is recommended to keep reconstituted reagents at room

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temperature for 15 minutes and then to mix them thoroughly but gently (no foam) with a Vortex mixer. The reconstituted standards and controls can be stored for 1 month at −20°C. Repeated freeze/thaw cycles have to be avoided.

Sample Preparation

Samples have to be diluted in Dilution Buffer (VP). For most of the determinations (serum samples, and no extreme values are expected) a dilution of 1:4 with Dilution Buffer VP should be suitable. According to expected vaspin levels the dilution with VP can be higher or lower.

Washing Buffer

The required volume of Washing Buffer is prepared by 1:20 dilution of the provided 20-fold concentrate with deionised water. The diluted Washing Buffer is stable for 4 weeks at 2-8°C. It has to be at room temperature for usage.

Analytical procedure for ELISA test
Assay procedure

When performing the assay, the Standards A-E, Control Sera KS1& KS2 and the samples should be pipetted as fast as possible (e.g., 15 minutes). To avoid distortions due to differences in incubation times, the Enzyme Conjugate EK as well as the succeeding Substrate Solution S should be added to the plate in the same order and in the same time interval as the samples. Stop Solution SL should be added to the plate in the same order as the Substrate Solution S.

For optimal results, accurate pipetting and adherence to the protocol are recommended.
1) Pipette in positions A1100 μl Dilution Buffer VP (blank).
2) Pipette in positions B1 100 μl of the Standard A (0.01ng/ml), pipette in positions C1 100 μl of the Standard B (0.075 ng/ml), pipette in positions D1 100 μl of the Standard C (0.2 pg/ml), pipette in positions E1 100 μl of the Standard D (0.5 ng/ml), pipette in positions F1 100 μl of the Standard E (1.0 ng/ml).

To control the correct accomplishment of the assay 100 μl of the 1:4 (or in respective dilution ratio of the samples) in Dilution Buffer VP diluted Control Sera KS1/KS2 can be pipetted in positions G1and H1

3) Pipette 100 μl each of the diluted samples (e.g. dilute 1:4 with Dilution Buffer VP) in the rest of wells, according to your requirements.

4) The wells was covered with sealing tape and incubate the plate for 1 hour at room temperature (shake at 350 rpm).

5) After incubation the contents of the wells were aspirated and washed the wells 5 times 300 μl Washing Buffer WP / well.

6) Following the last washing step pipette 100 μl of the Antibody Conjugate AK in each well.

7) Coverd the wells with sealing tape and incubate the plate for 1 hour at room temperature(shake at 350 rpm).

8) After incubation the contents of the wells were aspirated and washed the wells 5 times 300 μl Washing Buffer WP / well.

9) Following the last washing step pipette 100 μl of the Enzyme Conjugate EK in each well.

10) Cover the wells with sealing tape and incubate the plate for 1 hour at room temperature (shake 350 rpm).

11) After incubation the wells were washed 5 times with Washing Buffer WP as described in step 4.

12) Pipette 100 μl of the Substrate Solution S in each well.

13) The microtiter plate was incubated for 30 minutes in the dark at room temperature.

14) The reaction was stopped by adding 100 μl Stopping Solution SL to all wells.

15) The absorbance was measured within 30 minutes at 450 nm .

Calculation

- Plot the standard concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis.
- Using the mean absorbance value for each sample to determine the corresponding concentration from standard curve.

Table (3.2): Reference values of vaspin

Age [years]	Mean Males [ng/ml]	SD [ng/ml]	Mean Femals [ng/ml]	SD [ng/ml]
19-25	0.208	0.12	2.277	1.8
26-30	0.241	0.23	1.13	0.88
34-44	0.241	0.15	0.999	0.78
45-54	0.253	0.18	0.708	0.69
55-68	0.240	0.20	0.503	0.31

3.8.2 Determination of glycated hemoglobin in whole blood

Glycated hemoglobin was determined by the colorimetric determination of glycated hemoglobin in whole blood using Stanbio Kit, Texas-USA (Trivelli, Ranney & Lai, 1971).

Principle of glycated hemoglobin (HbA1c) test

A preparation of hemolyzed whole blood is mixed with a weakly binding cation exchange resin. The non-glycosylated hemoglobin (HbA0) binds to the resin, leaving HbA1c free to be removed by means of a resin separator in the supernate. The percent of HbA1c is determined by measuring the absorbance values at 415 nm of the HbA1c fraction and of the total Hemoglobin fraction, calculating the ratio of absorbance's (R), and comparing this ratio to that of a HbA1c standard carried through the same procedure. Results are expressed as HbA, but can be converted or derived as HbA1c by using a conversion factor or when using HbA1c value for the standard. See table (3.3) which illustrate the reagent used for glycated hemoglobin(HbA1c) test.

Table (3.3): Reagents used for glycated hemoglobin(HbA1c) test.

Glycated hemoglobin lon Exchange Resin. Each tube contains 3.0 mL cation exchange resin 8 mg/dL. pH 6.9
Glycated hemoglobin Lysing Reagent Contains potassium cyanide 10 mmol/L and surfactants.
Glycated hemoglobin Standard (Lyophilized) (1 vial) Prepared from packed human erythrocytes.

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Procedure for glycated hemoglobin(HbA1c) test.

Hemolysate Preparation

1. Pipette 500 µl Lysing reagent into tubes labeled Standard (S),Unknown (U) and Control (C).

2. Pipette100 µl of each well-mixed blood sample into appropriately labeled tube and mix.

3. Allow to stand for 5 minutes at room temperature (15-30°C) to complete hemolysis.

Glycated hemoglobin separation and assay

1. Label resin tubes Standard (S), Unknown (U) and Control (C).

2. Pipette I00 µl of the prepared hemolysate into appropriately labeled resin tube.

3. Position a resin separator in the tube so rubber sleeve is approximately 1-2cm above liquid level.

4. Mix tubes on a hematology rocker for 5 minutes. Alternatively tubes may be mixed by hand if held above the resin.

5. At the end of the 5 minute mixing, push resin separator into tube until resin is firmly packed in bottom of the 13mm tube.

6. Pour each supernate directly into separate cuvettes for absorbance measurements.

7. Read absorbance (Agly) of Standard, Unknown and Control vs. water at 415 nm within 60 minutes.

Total hemoglobin assay

1. Pipette 5.0 mL deionized water into tubes labeled Standard (S), Unknown (U) and Control (C).

2. Pipette 20 µl of hemolysate into appropriately labelled tube.

Mix well and transfer to cuvette for absorbance reading.

3. Read absorbance (Atot) of Standard, Unknown and Control vs. water at 415nm within 60 minutes.

Calculation

For each Standard and Unknown calculate the ratio (R) of the glycated hemoglobin absorbance to the hemoglobin absorbance as follows:

$$(R) = Agly / Atot$$

Hemoglobin (%) = <u>(R) Unknown x Hemoglobin Standard (%)</u>

(R) Standard

Results may also be reported as HbA1c when compared to the reference A1c method, the Stanbio method showed a 98% correlation with an equation of:

$$Y \text{ (A1c value)} = 0.838 \times (\text{Stanbio value}) - 0.732$$

The value obtained by the Stanbio method may be converted to Calculated A1c value by use of this formula. For a direct calculated A1c value, the value of the standard may be changed to 7.6% in lieu of the 10.0% and the results will be A1c values.

3.8.3 Determination of serum glucose

Serum glucose was determined by glucose-oxidase procedure (Trinder, 1969) using labkit reagent kits. See table (3.4)

Principle of serum glucose test

Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconic acid. The formed hydrogen peroxidase (H2O2) is detected by chromogenic oxygen acceptor, phenol aminophenzone in the presence of peroxydase (POD).

GOD

$$\beta\text{-D -Glucose} + 2H_2O + O_2 \rightarrow \text{Gluconic acid} + H_2O_2$$

POD

$$H_2O_2 + 4\text{-AP} + \text{Phenol} \rightarrow \text{Quinone} + H_2O$$

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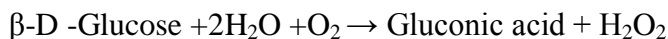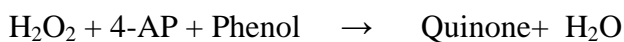
Table (3.4): Reagents composition of glucose kit

Reagent	Concentration
TRIS (pH 7.4)	92 mmol/l
Phenol	0.3mmol/l
4-Aminophenzone (4-AP)	2.6 mmol/l
Glucose oxidase (GOD)	15000 U/L
Peroxidase (POD)	1000 U/L
Standard	100 mg/dl

Assay procedure

For glucose determination a500 nm wave length was used with 1cm optical path at 37 °C incubation the measurement were taken against blank as described below. Adjust the instrument to zero with blank of reagent as illustrated in the following table

	Blank	standard	test
Reagent(ml)	1.0	1.0	1.0
Standard(µl)	----	10	----
Sample(µl)	----	----	10

Mix and incubate for 10 minutes at 37 °C

Read the absorbance (A) of sample and standard against blank. The colour is stable at least 30 minutes.

For calculation of blood glucose levels, the following formula was applied and results were compared to reference value in table 3.5.

Glucose [mg / dl] = $\dfrac{\Delta A \text{ sample} \times \text{ concentration of standard}}{\Delta A \text{ standard}}$

Conversion factor : mg/dl $\times$ 0.0555 = mmol/L

Table (3.5): Reference value (fasting glucose)

(Palestinian Clinical Laboratory Tests Guide, PCLTG, 2005)

Child	60 – 100 mg/dl
Adult	70 – 110 mg/dl

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3.8.4 Determination of serum insulin

Serum insulin was determined by using DRG insulin ELISA assay (Flier, Bleich, Boro, Kahn & Roth, 1979)

Principles of the test:

The DRG insulin ELISA kit is solid phase enzyme linked immunoassay (ELISA) based on sandwich principle, the microtiter wells are coated with monoclonal antibody directed toward a unique antigenic site on the insulin molecule. An aliquot of patient sample containing endogenous insulin is incubated in coated well with enzyme conjugate, which is an anti-insulin antibody conjugated with biotin. After incubation the unbounded conjugate is washed off. During the second incubation step streptavidin peoxidase enzyme complex bind to the biotin anti insulin antibody. The amount of bound HRP complex is proportional to the concentration of insulin in the sample. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of insulin in patient sample.

Table (3.6): Reagents composition of insulin ELISA kit

Microtiterwells 96 well coated with anti-insulin antibody (monoclonal)	$1\times12\times8$
Zero standard 1 vial	$1\times3ml$
Standard (1-5) 5 vials 1ml ready to use	$5\times1ml$
Enzyme conjugate 1 vial	$1\times5ml$
Enzyme complex 1 vial	$1\times7ml$
Substrate solution 1 vial	1×14 ml
Stop solution 1 vial	14ml
Wash solution 1 vial (40x concentrated)	30ml

Reagent preparation

Bring all reagent and required number of strip to room temperature prior to use

Wash solution

Add deionized water to 40 x concentrated wash solution.

Test procedure

1. Secured the diserd number of microtiter wells in frame holder .
2. Dispensed 25µl of each standard, control, and sample with new disposable tips into appropriate wells.
3. Dispensed 25µl of enzyme conjugate into each well.
4. Incubated 30 minute at room temperature .
5. Briskly shake out the content of the well. Rinse 3 time with diluted wash solution. Strike the wells sharply on absorbent paper to remove residual droplet.
6. Add 50µl of enzyme complex to each well .
7. Incubated 30 min at room temperature .
8. Briskly shaked out the content of the well. Rinse 3 time with diluted wash solution, striked the wells sharply on absorbent paper to removed residual droplet.
9. Add 50µl of substrate to each well .
10. Incubated 15 min at room temperature.
11. Stopped the enzymatic reaction by adding 50µl of stop solution to each well.
12. Determined the absorbance of each well at 450 nm with microtiter reader.

Calculation of result

Using linear graph paper, construct a standard curve by blotting the mean absorbance obtain from each standard a giants its concentration with absorbance value on vertical Y axis and concentration on horizontal X axis.

Using the mean absorbance value for each sample determine corresponding concentration from standard curve.

Expected normal values for insulin:

2-25 µIU/ml.

3.8.5 Determination of HOMA-IR

Insulin resistance was estimated by Homeostasis Model Assessment of Insulin Resistance (Matthews et al., 1985 and Levy et al., 1988).

$$HOMA\text{-}IR = \frac{(fasting\ insulin\ (\mu IU/ml)\ X\ fasting\ glucose\ mmol/l)}{22.5}$$

Normal range

Normal insulin resistance   < 3.

Moderate insulin resistance   Between 3 and 5.

Severe insulin resistance   >5.

3.8.6 Determination of serum cholesterol

Enzymatic colorimetric method was used for the quantitative determination of total cholesterol in serum or plasma, using AMS kit (Meiattini, Prencipe, Bardelli, Giannini & Tarli, 1978).

Principle

The measurement is based on the following enzymatic reaction

$$Cholesterol\ ester + H2O \xrightarrow{CHE} cholesterol + fatty\ acid$$

$$Cholesterol + O_2 \xrightarrow{CHOD} cholest\text{-}4\text{-}en\text{-}3\text{-}one + H_2O_2$$

$$2H_2O_2 + 4\text{-}aminoantipyrine + hydroxybenzoate + Phenol \xrightarrow{POD} red\ complex + 4\ H_2O$$

30
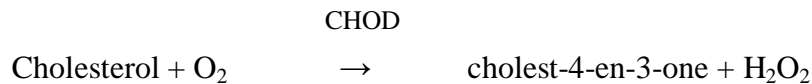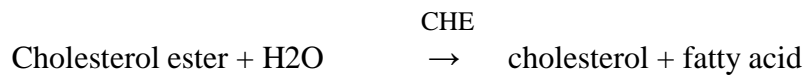
Table (3.7): Reagents composition of cholesterol kit

Concentration are those in final mixture.

Reagent	Concentration
Good's buffer (pH 6.7)	50 mmol/l
4- Aminoantipyrine	0.3mmol/l
Cholesterol esterase (CHE)	≥ 300 U/l
Cholesterol oxidase (CHO)	≥ 100U/l
Peroxidase (POD)	≥ 500 U/l
Hydroxbenzoic cid	12 mmol/l
Sodioazide	≤0.095g/l
Standard	200 mg/dl

Assay procedure

For cholesterol determination a510 nm wave length was used with 1cm optical path at 37 ℃ incubation the measurement were taken against blank as described below. Allow the reagent to reach working temperature before using .

Pipette into disposable or well clean cuvettes.

	Blank	Standard	Sample
Reagent	1000µl	1000µl	1000µl
Standard		10 µl	
Sample			10 µl

Mix and incubate for 10 minutes at 37℃. Read the absorbance (A) of standard and samples at 510 nm against blank. Colour is stable for 60 minute, protected from light.

For calculation of cholesterol in serum the following formula was used and results were compared with the reference value in table 3.8

$$\text{Cholesterol (mg/dl)} = \frac{\Delta A \text{ sample X concentration of standard}}{\Delta A \text{ standard}}$$

Table (3.8): Reference values for cholesterol concentration in seurm

Recommended value	< 200 mg/dl
Upper limits	200-239 mg/dl
High values	>240 mg/dl

3.8.7 Determination of serum triglycerides

Enzymatic colorimetric method was used for the quantitative determination of triglycerides in serum or plasma, using cromatest kit (Bucolo and David, 1973).

Principle of serum triglycerides test:

The method is based on enzaymetic hydrolysis of serum or plasma triglyceride to glycerol and free fatty acid by lipoprotein lipase (LPL). The glycerol is phosphlorylated by adenosin triplphosphate in the presence of Glycerolkinase (GK) to form glycerol -3-phosphate (G-3-P) and adenosine diaphosphate (ADP). G-3-P is oxidized by glycerolphosphate oxidase (GPO) to form dihydroxyaceton phosphate (DHAP) and hydrogen peroxide. A red chromgen is produced by peroxidase (POD) catalyze coupling of 4–aminoantipyrine (4-AA) and phenol with hydrogen peroxide (H_2O_2), proportional to concentration of triglyceride in sample

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{fatty acid}$$

$$\text{Glycerol} + ATP \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate} + ADP.$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GP}} \text{Dihydroxyaceton phosphate} + H_2O_2.$$

$$H_2O_2 + 4\,\text{Phenol} + 4\text{-AA} \xrightarrow{\text{POD}} \text{Quinoneimine} + H_2O.$$

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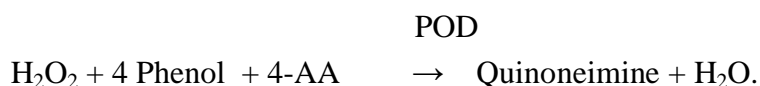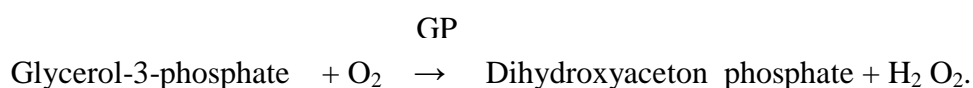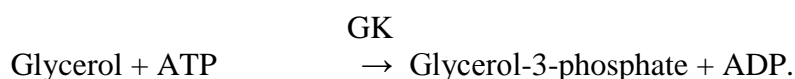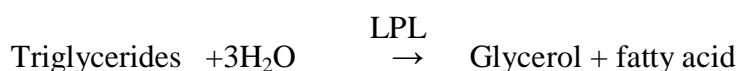
Table (3.9): Reagents composition of triglycerides kit

Concentrations are those in the final test mixture.

Reagent	Concentration
Cood's buffer (6.8)	50 mmol/l
ATP	2 mmol/l
Phenol	3 mmol/l
Glycerokinase (GK)	≥ 1 KU/I
Peroxidase(POD)	≥ 2.5 KU/I
Lipoprotein lipase((LPL)	≥ 12 KU/I
4-Aminoantipyrine	0.5 mmol/l
Glycerol-3-phosphate-oxidase (GPO)	≥ 10 KU/I
4-AA	0.5 mmol/L
Non ionic tensioactive	2 g/L(w/v)
Standard	200 mg/dl

Assay Procedure

For triglycerides determination a500 nm wave length was used with 1cm optical path at 37 ºC incubation the measurement were taken against blank as described below.

	Blank	standard	Sample
Reagent	1000µl	1000µl	1000µl
Standard		10 µl	
Sample			10 µl

Mix and incubate for 5 minutes at 37°C. Read the absorbance (A) of standard and samples at 500 nm against blank. Colour is stable for 60 minute, protected from light.

For calculation of triglycerides in serum the following formula was used and results were compared with the reference value in table 3.10

Triglycerides [mg / dl] = $\dfrac{\Delta A \text{ sample X concentration of standard } \Delta A}{\text{standard}}$

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Table (3.10): Reference values for seurm triglycerides

Triglyceride	Risk classification
< 150 mg/dl	Normal
150-199 mg/dl	Borderline high
200-499 mg/dl	High
≥ 500 mg/dl	Very high

3.8.8 Determination of serum high density lipoprotein cholesterol

Liquid high density lipoprotein cholesterol (HDL-C) precipitant was used for the determination of HDL-C Cholesterol using Diasys Diagnostic Systems, Germany (Grove, 1979).

Principle

Chylomicrons, VLDL-C and LDL-C were precipitated by adding phosphotungstic acid and magnesium ions to the sample. Centrifugation leaves only the HDL-C in the supernatant, their cholesterol content is determined enzymatically using cholesterol reagent. See table (3.11).

Table (3.11): Reagents used for determination of HDL-C

Reagent	Concentration
Monoreagent contain: Magnesium chloride.	1.4 mmol/l
Phosphotungstic acid.	8.6 mmol/l
Cholesterol standard.	200 mg/dl

Assay procedure

1- Precipitation

- 100 µl of standard (sample or control) were added to 100 µl of the precipitation reagent and mixed well.

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- The mixture was allowed to stand for 15 min at room temperature, and then centrifuged for 20 min at 4000 rpm.

2- Cholesterol determination

For cholesterol determination a500 nm wave length was used with 1cm optical path at 37 ℃ incubation the measurement were taken against blank as described below.

- 100 µl of the supernatant of standard (sample or control) was added to 1ml of the cholesterol reagent and mixed well.
- The mixture was incubated for 5 min at 37 ℃.
- The absorbance was measured within 45 min.

For calculation of HDL-C in serum the following formula was used and results were compared with the reference value in table 3.12

$$\text{HDL-C (mg/dl)} = \frac{\Delta A \text{ sample X concentration of standard } \Delta A}{\text{standard}}$$

Table (3.12): Reference value for HDL-C

Child	37 – 75 mg/dl
Adult: M	35 – 65 mg/dl
F	35 – 80 mg/dl

3.8.9 Determination of serum low density lipoproteins cholesterol

Serum low density lipoproteins cholesterol (LDL-C) was calculated using the empirical relationship of Friedewald (Grove, 1979).

Principle:

The ultracentrifuge 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-C and plasma triglycerides (TG) using the empirical relationship of Friedewald.

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$$\text{LDL-C} = \text{Total Cholesterol} - (\text{HDL-C} + \text{TG/5})$$

3.8.10 Determination of alanine aminotransferase activity

Serum ALT activity was measured by using optimized ultraviolet-test according to International Federation of Clinical Chemistry and Laboratory Medicine (Thomas, 1998) using cromatest reagent kit.

Principle

$$\text{L- Alanine + 2-Oxoglutarate} \xleftrightarrow{\text{ALT}} \text{L- Glutamate + Pyruvate}$$

$$\text{Pyruvate + NADH + H}^+\text{D -} \xleftrightarrow{\text{LDH}} \text{Lactate + NAD}^+$$
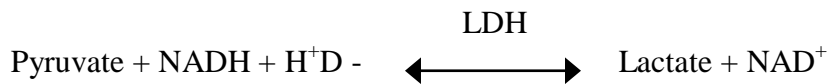
Reagents

Reagent	Components	Concentrations
Reagent 1	TRIS pH 7.3	150 mmol/l
	L- Alanine	750 mmol/l
	LDH (lactate dehydrogenase)	$\geq$ 1350 U/l
Reagent 2	2-Oxoglutarate	75 mmol/l
	NADH	1.3 mmol/l

Substrate start

The reagents are ready to use.

Reagent preparation

Mix 4 parts of R1 with 1 parts of R2, (e.g. 20 ml R1 + 5 ml R2) = monoreagent. Stability: 4 weeks at 2-8 $^{\circ}$C. The monoreagent must be protected from light.

Procedure:

Pre incubate working reagent, sample, and control, to reaction temperature.
Set the photometer to 0 absorbance with distilled water .
Pipette into a cuvette.

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Reaction temperature	37°C
Working reagent	1000μl
Sample	50μl

Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch, incubate for 1 min and record initial absorbance reading. Repeat the absorbance reading exactly after 1, 2 and 3 min. Calculate the difference between absorbance's. Calculate the mean of the result to obtain the average change in absorbance per minute (Δ A/min).

For calculation of ALT activity at 37°C the following formula was used and the result were compared with reference range illustrated in table 3.12.

U/L = ΔA/min x 3333(37°C) = ALT activity [U/L]

Table (3.13): Reference range of ALT activity in serum

Adult	37 °C	Up to 40 U/L

3.8.11 Determination of aspartate aminotransferase activity

Serum AST activity is measured by using optimized ultraviolet-test according to International Federation of Clinical Chemistry and Laboratory Medicine (Thomas, 1998) using Cromatest reagent kit.

Principle

$$\text{L- Asprtate} + \text{2-Oxoglutarate} \xrightarrow{\text{AST}} \text{L- Glutamate} + \text{Oxalacetate.}$$

$$\text{Oxalacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{L} - \text{Malate} + \text{NAD}^+.$$
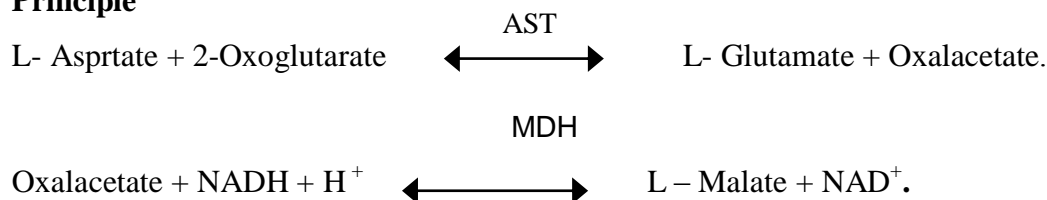
Table (3.14): Illustrated the reagents used for measurement of ALT activity.

Reagent	Components	Concentrations

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Reagent 1 AST substrate	TRIS pH 7.8	121mmol/l
	L- Aspartate	362 mmol/l
	MDH (Malate dehydrogenase)	≥460 U/l
Reagent 2	2-Oxoglutarate	75 mmol/l
	NADH	1.3 mmol/l

Reagent preparation

Mix four parts of R1 with one part of R2, (e.g. 20 ml R1 + 5 ml R2) = monoreagent. The monoreagent is Stable for four weeks at 2-8 ºC and must be protected from light.

Procedure:

Preincubate working reagent, sample, and control, to reaction temperature.
Set the photometer to 0 absorbance with distal water.
Pipette into acuvette:

Reaction temperature	37°C	37°C
Working reagent	1000µl	1000 µl
Sample or control	50 µl	50 µl
Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch, incubate for 1 min and record initial absorbance reading. Repeat the absorbance reading exactly after 1, 2 and 3 min. Calculate the difference between absorbance's. Calculate the mean of the result to obtain the average change in absorbance per minute (Δ A/min).		

For calculation of AST activity at 37°C the following formula was used and the result were compared with reference range illustrated in table 3.13.

U/L = ΔA/min x 3333(37°C) = AST activity [U/L]

Table (3.15): Reference range for AST activity

Adult	37 °C	Up to 40 U/L

3.8.12 Determination of serum urea

Serum urea was determined by using U.V test (Fawcett & Scott, 1960) using AMS reagent kits.

Principle

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_4 + H_2CO.$$

$$2\text{-ketoglutarate} + NH_4 + NADH \xrightarrow{\text{GLDH}} \text{L-Glutamate} + NAD^+ + H_2O$$
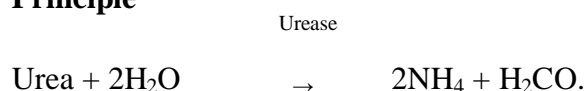
The decrease in NADPH absorbance is proportional to urea level in the sample.

Table (3.16): Reagents used in determination of serum urea.

Reagent	Concentration
A : TRIS PH (7.8)	150 mmol/l
2- ketoglutarate	8.75mmol/l
ADP	0.75 mmol/l
Urease	≥ 7.5 ku/l
GLDH	≥ 1.25 ku/l
Sodium azide	≤ 0.95g/l
B: NADH	1.32 mmol/l
Sodium azide	≤ 0.95g/l
Standard	50 mg/dl

Assay procedure

The working solution was prepared by mixing 4 parts of A with 1 part of B to obtain working reagent

Let stand working reagent at least 30 minute at room temperature before used.

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Analytical procedure :

For urea determination a340 nm wave length was used with 1cm optical path at 37 ℃ incubation the measurement were taken against blank as described below.

Reaction: Fix time (decrease).

Monoreagent procedure :

	Blank	Standard	Sample
Working reagent	1000µl	1000µl	1000µl
Standard	--	10 µl	--
Sample	--	--	10 µl
Mix, incubate 30 second at 37 °C, than read A1of sample, standard, blank, after precisely 60 seconds read absorbance A2 Determine : $\Delta A = ((A1-A2)\text{sample or standard}) - ((A1-A2)\text{BLANK})$			

Calculation

$\Delta A = (A1 - A2)$ sample or standard

Urea (mg/dl) = $\dfrac{\Delta A \text{ sample X concentration of standard}}{\Delta A \text{ standard}}$

Reference value

(PCLTG, 2005)

Child	5 - 30 mg/dl
Adult	13 - 43 mg/dl

3.8.13 Determination of serum creatinine

Serum creatinine was determined by using Jaffa's kinetics method (Newman & Price, 1999) using coral reagent kits.

Principle:

Pricric acid in alkaline medium react with creatinine to forms orange coulour complex with the alkaline picrate. Intensity of colour formed during the fixed time is directly proportional to amount of creatinine present in the sample.

Creatinine + alkaline Picrate → orange coloured complex

Reagents

Reagent
L1: picric acid reagent
L2: buffer reagent
S: Creatinine Standard(2mg/dl)

Working reagent

The working solution was prepared by mixing equal volumes picric acid reagent and buffer reagent, the working reagent is stable at R.T (25-30°C) for at least one week.

Procedure:

Wavelength: 520 nm.

light path: 1cm

Temperature: 30 ℃ /37 ℃

Addition sequence	Blank	Standard	Test
L1 reagent	0.5ml	0.5ml	0.5ml
L2 reagent	0.5ml	0.5ml	0.5ml
Standard		100µl	
Sample			100µl

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Mix well and read initial absorbance A1 for standard and test after exactly 30 seconds. Read another absorbance A2 of standard and test exactly after 60 seconds later. Calculate the change in absorbance ΔA for both the standard and test.

For standard   ΔAS =A2-A1

For Test   ΔAT =A2-A1

Calculation.

Creatinine (mg/dl) = $\dfrac{\Delta A \text{ sample X concentration of standard } \Delta A}{\text{standard}}$

Reference value (in serum) (PCLTG, 2005)

Infant	0.2 – 0.4 mg/dl
Child	0.3 - 0.7 mg/dl
Adolescent	0.5 - 1.0 mg/dl
Adult: M	0.6 - 1.2 mg/dl
F	0.5 -1.1 mg/dl

3.9   Statistical analysis

Data were computer analyzed using SPSS/ PC (Statistical Package for the Social Science Inc. Chicago, Illinois USA, statistical package version 22.0)

- Simple distribution of the study variables and the cross tabulation were applied.
- Chi-square (χ^2) was used to identify the significance of the relations, associations, and interactions among various variables. Yates's continuity correction test, $\chi^2_{(corrected)}$, was used when not more than 20% of the cells had an expected frequency of less than five and when the expected numbers were small.

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- The independent sample t-test procedure was used to compare means of quantitative variables by the separated cases into two qualitative groups such as the relationship between cases and controls vaspin.
- Pearson's correlation test was applied.
- The results in all the above mentioned procedures were accepted as statistical significant when the p-value was less than 5% ($p<0.05$).
- Range as minimum and maximum values was used.
- The percentage difference was calculated according to the formula: Percentage difference equals the absolute value of the change in value, divided by the average of the 2 numbers, all multiplied by 100.

  Percent difference = $| (V1 - V2) | / ((V1 + V2)/2)) \times 100$

- SPSS program version 22.0 was also used for correlation graph plotting between vaspin and other studied parameters as well as for chart graphs plotting.

Chapter 4
Results

Chapter 4
Results

4.1 Personal profile of the study population

Table 4.1 summarizes personal profile of the study population. The study included 88 cases (44 males and 44 females) and 88 controls (44 males and 44 females). Age classification showed that 41 (46.6%) cases and 42 (47.7%) controls were 34-44 years old. Age group 45-54 years comprised 31 (35.2%) cases and 30 (34.1%) controls. Cases and controls aged 55-68 years old were 16 (18.2%) and 16 (18.2%), respectively. The difference between cases and controls in term of age distribution was not significant (χ2=0.028, P=0.986). The mean ages of cases and controls were 45.9±7.1and 45.8±7.0 years old, respectively. The independent sample t-test also showed no significant difference between mean ages of cases and controls (t=0.000, P=1.000). Analysis of the educational status of the study population showed that 33 (37.5%) cases and 49 (55.7%) controls had finished university degree, 24 (27.3%) cases and 22 (25.0%) controls had finished secondary school, 14 (15.9%) cases and 6 (6.8%) controls finished preparatory school, 10 (11.4%) cases and 8 (9.1%) controls had passes primary school, and 7 (7.9%) cases and 3 (3.4%) control were illiterate. The difference between various educational levels of cases and controls was not significant ($\chi^2_{corrected}$ =6.171, P=0.187).

Table (4.1): Personal profile of the study population

Personal profile	Cases (n=88)		Controls (n=88)		Test		P-value
	No.	%	No	%			
Age (Year) 34-44 45-54 55-68	41 31 16	46.6 35.2 18.2	42 30 16	47.7 34.1 18.2	χ^2	0.028	0.986
Mean ±SD	45.9±7.1		45.8±7.0		t	0.000	1.000
Gender Male Female	44 44	50 50	44 44	50 50	χ^2	0.000	1.000
Education University secondary school Preparatory school primary illiterate	33 24 14 10 7	37.5 27.3 15.9 11.4 7.9	49 22 6 8 3	55.7 25.0 6.8 9.1 3.4	χ^2	6.171	0.187*

* P value of $\chi^2_{(corrected)}$ test, P>0.05: Not significant

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4.2 Socioeconomic data of the study population

Socioeconomic data of the study population is provided in Table 4.2. The employed cases and controls were 43 (48.9%) and 47 (53.4%) whereas 45 (51.1%) cases and 41 (46.6%) controls were unemployed. The difference between the two groups was not significant (χ^2=0.364 P=0.546). Family income\month also showed no significant difference between cases and controls (χ^2=1.225, P=0.542). Regarding family history of diabetes, 57 (64.8%) cases and 26 (29.5%) controls reported that they had family history of diabetes whereas 31 (35.2%) cases and 62 (70.5%) controls did not (χ^2=21.912, P=0.000), indicating that family history is associated with diabetes. In addition, 23 (26.1%) cases were smokers compared to 14 (15.9%) controls (χ^2=2.772, P=0.096).

Table (4.2): Socioeconomic data of the study population

Socioeconomic data	Cases (n=88)		Controls (n=88)		χ2	P-value
	No.	%	No.	%		
Employment. Yes No	43 45	48.9 51.1	47 41	53.4 46.6	0.364	0.546
Family income/month. (NIS)* <1000 1000-2000 >2000	43 8 37	48.9 9.1 42.0	36 8 44	40.9 9.1 50	1.225	0.542
Family history of diabetes. Yes No	57 31	64.8 35.2	26 62	29.5 70.5	21.912	0.000
Smoking. Yes No	23 65	26.1 73.9	14 74	15.9 84.1	2.772	0.096

*NIS: New Israeli Shekels
P<0.05:Significant, P>0.05:not significant.

4.3   Physical activity and meal frequency/day among the study population

Table 4.3 illustrates physical activity and meal frequency/day among the study population. Although the number of cases who have physical activity 54 (61.4%) was higher than controls 42 (47.7%), the difference between the two groups was not significant (χ^2=3.300 and P=0.069). Meal frequency/day indicated that 4 (4.5%), 26 (29.5%), 40 (45.5%) and 18 (20.5%) cases ate 1,2,3 and ≥4 meals/day compared to 2 (2.3%), 32 (36.4%), 47 (53.4%) and 7 (7.9%) controls ($\chi^2_{corrected}$ =5.011 and P=0.171).

Table (4.3): Physical activity and meal frequency/day of the study population.

Item	Cases (n=88)		Controls (n=88)		χ^2	P- value
	No.	%	No.	%		
Physical activity						
Yes	54	61.4	42	47.7	3.300	0.069
No	34	38.6	46	52.3		
Meal frequency/day						
One	4	4.5	2	2.3	5.011	0.171*
Two	26	29.5	32	36.4		
Three	40	45.5	47	53.4		
Four and more	18	20.5	7	7.9		

*P-value of $\chi^2_{(corrected)}$ test, P>0.05: Not significant.

4.4   Duration of diabetes and its distribution among patients

The distribution of diabetic patients by the duration of the disease is demonstrated in Table 4.4. Patients with diabetes ≤ 5 years were 45 (51.1%), whereas those with diabetic duration of 6-11 years were 26 (29.6%). The rest of patients 17 (19.3%) had diabetes for more than 11 years.

Table (4.4): Distribution of diabetic patients (n=88) by the duration of the disease.

Duration of diabetes (Year)	N	% of cases
≤ 5 years	45	51.1
6-11years	26	29.6
>11 years	17	19.3

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4.5 Self-reported complications among the study population

The main self-reported complications among the study population are summarized in Table 4.5. The percentages of retinopathy and cardiovascular disease were significantly higher in cases compared to controls (21.6 and 18.2% *versus* 3.4 and 2.3%, $\chi^2_{(corrected)}$=11.688, P=0.000 and $\chi^2_{(corrected)}$= 10.459, P=0.001, respectively). Similarly the percentage of neuropathy was significantly higher in cases than controls (10.2% *versus* 1.1 %, $\chi^2_{(corrected)}$=5.195, P=0.023).

Table (4.5): Self-reported complications among the study population.

Self-reported complications	Cases (n=88)		Controls (n=88)		χ^2	P-value*
	n	%	n	%		
Retinopathy						
Yes	19	21.6	3	3.4	11.688	0.000
No	69	78.4	85	96.6		
Cardiovascular disease						
Yes	16	18.2	2	2.3	10.459	0.001
No	72	81.8	86	97.3		
Neuropathy						
Yes	9	10.2	1	1.1	5.195	0.023
No	78	88.6	87	989		

*P-value of $\chi^2_{(corrected)}$ test.
P<0.05: Significant.

4.6 Body mass index of the study population

Table 4.6 shows BMI of the study population. The mean weight of cases was78.3±12.8 kg compared to 77.9±16.6 kg of controls (% difference=0.5, t=0.183 and P=0.855). Similarly there was no significant difference in the mean height between cases and controls (166.9±8.7 *versus* 166.6±8.2 cm, %difference=0.2, t=0.175 and P=0.862). Therefore, the difference of BMI between cases and controls was not significant (28.4±5.1 *versus* 28.0±6.0, % difference=1.4, t =0.283 and P=0.778).

Table (4.6): Body mass index (BMI) of the study population

Anthropometric measurement	Cases (n=88) mean± SD	Controls (n=88) mean± SD	% difference	t	P-value
Weight (kg)* (min-max)	78.3±12.8 (52-115)	77.9±16.6 (48-105)	0.5	0.183	0.855
Height (cm)** (min-max)	166.9±8.7 (150-188)	166.6±8.2 (145-183)	0.2	0.175	0.862
BMI**(Kg/m^2** (min-max)	28.4±5.1 (19.7-39.3)	28.0±6.0 (19.1-46.2)	1.4	0.283	0.778

*Kg: kilogram, **cm: centimeter. ***BMI (Kg/m^2): 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 BM≥30.0 were considered obese **(WHO, 2012).** P>0.05: not significant.

4.7 Serum vaspin, insulin, HbA1c, glucose and HMOA-IR level of the study population

As indicated in table 4.7, and figure 4.1, there was a significant increase in the mean level of vaspin in cases compared to controls (0.73±0.67 *versus* 0.47±0.43ng/ml, % difference=43.3, t=2.161 and P=0.033). Serum insulin was not significantly higher in cases compared to controls (17.4±11.6 *versus* 15.2±8.4 μlU/ml, % differences=13.5, t=1.001 and P=0.320). Table 4.7, also showed that the mean level of whole blood HbA1c was significantly higher in cases than in controls (6.7±1.3*versus* 4.7±0.65 %, % differences=35.1, t=9.260 and P=0.000). Similarly, serum glucose was significantly elevated in cases compared to controls (189.6±79.1 *versus* 90.7±13.6 mg/dl, % differences=70.5, t=8.165 and P=0.000). However, HOMA-IR showed significant increase in cases with respect to controls (8.0±7.3 veusus 3.6±2.4 % ,% differences=75.9, t=3.775 and P=0.000).

Table (4.7): Serum vaspin, HbA1c, glucose, insulin levels, and HMOA-IR of the study population.

Parameter	Case (n=88) mean ±SD	Control (n=88) mean ±SD	% difference	t	P-value
Vaspin (ng/ml) (min-max)	0.73±0.67 (0.1-2.4)	0.47±0.43 (0.16-1.44)	43.3	2.161	0.033
HbA1c (%) (min- max)	6.7±1.3 (4.1-10.0)	4.7±0.65 (3.5-5.6)	35.1	9.260	0.000
Glucose (mg/dl) (min- max)	189.6±79.1 (82-417)	90.7±13.6 (71-134))	70.5	8.165	0.000
Insulin (µlU/ml) (min-max)	17.4±11.6 (3.9-49.7)	15.2 ±8.4 (4.4-42.2)	13.5	1.001	0.320
HOMA-IR (%) (min-max)	8.0±7.3 (1.18-40.5)	3.6±2.4 (0.96-11.9)	75.9	3.775	0.000

P<0.05: Significant.
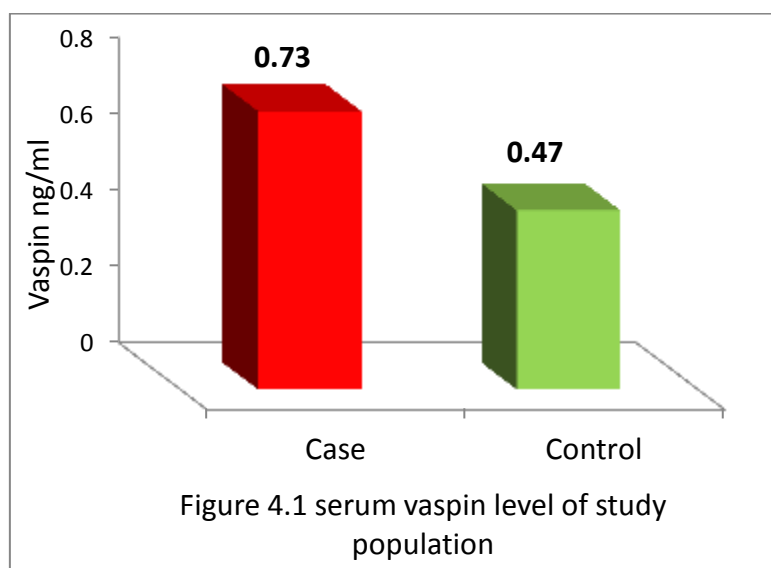
Figure 4.1 serum vaspin level of study population

Figure (4.1): Seurm vaspin level of the study population.

4.8 Serum lipid profile of the study population

Table 4.8 demonstrated serum lipid profile including cholesterol, triglycerides, HDL-C and LDL-C of the study population. The mean level of triglycerides was found to be significantly higher in cases compared to controls (187.6±133.8 *versus* 133.7±71.8 mg/dl, % of differences=33.6, t=2.356 and P=0.021). In contrast, HDL-C was significantly lower in cases than in controls (45.3±10.3 *versus* 50.8±13.8 mg/dl, % difference=-11.4, t=2.094 and P=0.039). On the other hand, the differences in the mean levels of cholesterol and LDL-C between cases and controls were not significant (P>0.05).

Table (4.8): lipid profile of the Study population.

Lipid profile	Case (n=88) mean ±SD	Control (n=88) mean ±SD	% difference	t	P-value
Cholesterol (mg/ dl) *(min-max)*	217.6±46.9 (122-322)	209.9±46.7 (122-325)	3.6	0.768	0.445
Triglycerides (mg/dl) (min-max)	187.6±133.8 (33-725)	133.7±71.8 (35-376)	33.6	2.356	0.021
HDL-C *(mg/dl) *(min-max)*	45.3±10.3 (27-70)	50.8±13.8 (28-91)	-11.4	2.094	0.039
LDL-C**(mg/dl) (min-max)	132.0±39.3 (45-230)	129.4±57.3 (49-231)	2.0	0.250	0.803

*HDL-C: High density lipoprotein cholesterol, **LDL-C: Low density lipoprotein cholesterol. P<0.05: Significant, P>0.05: Not significant.

4.9 Liver and kidney functions of the study population

The activities of serum ALT and AST as markers of liver function as well as serum urea and creatinine concentrations as indicators of kidney function are pointed out in Table 4.9. There were no significant increases in ALT and AST activities in cases compared to controls (22.7±9.9 and 24.4±10.8U/L *versus* 20.2±7.8 and 24.1±11.2 U/L, % difference=11.7 and 1.2 and P=0.198 and 0.878, respectively). Conversely, there were significant elevations in urea and creatinine concentrations in cases compared to controls (24.9±12.4 and 0.81±0.3 *versus* 20.3± 7.0 and 0.64±0.2 mg/dl, % of differences 20.4 and 23.4, P=0.036 and 0.002, respectively).

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Table (4.9): Liver and kidney functions of the study population.

	Case (n=88) Mean ±SD	Control(n=88) Mean ±SD	% Difference	t	P-value
ALT(U/L) (min-max)	22.7±9.9 (11-48)	20.2±7.8 (9-41)	11.7	1.298	0.198
AST(U/L) (min-max)	24.4±10.8 (11-55)	24.1± 11.2 (11-58)	1.2	0.155	0.878
Urea(mg/dl) (min-max)	24.9±12.4 (10-72)	20.3± 7.0 (10-44)	20.4	2.128	0.036
Creatinine (mg/dl) (min-max)	0.81±0.3 (0.36-1.59)	0.64±0.2 (0.3-1.10)	23.4	3.162	0.002

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase.

P<0.05: Significant and P>0.05: Not significant.

4.10 Comparison between different parameters in non-obese cases and non-obese controls.

4.10.1 Vaspin, HbA1c, glucose, HOMA-IR, insulin and BMI.

Although the mean levels of vaspin was higher in non obese cases compared to non obese controls, the difference was not significant (0.48±0.42 *versus* 0.33±0.27ng/ml, P=0.188, table 4.10. Figure 4.2). On the other, HbA1c and glucose showed significant increase in non obese cases compared to non obese controls groups. (6.7±0.94 and 198.2±82.9 *versus* 4.6±0.61 and 87.0±8.8, P=0.000 and P=0.000). Insulin, HOMA-IR and BMI showed no significant difference between non obese cases and non obese controls (P>0.05).

Table (4.10): Comparison between vaspin, insulin, HbA1c, glucose, HOMA-IR and BMI in non-obese cases and non-obese controls.

Parameter	Non Obese Case (n=22)	Non Obese Control (n=22)	t	P-value
	Mean ± SD			
Vaspin(ng/ml)	0.48±0.42	0.33±0.27	1.337	0.188
HbA1c(%)	6.7±0.94	4.6±0.61	8.599	0.000
Glucose(mg/dl)	198.2±82.9	87.0±8.8	6.258	0.000
Insulin(µIU/ml)	11.0±9.99	11.9±5.2	0.374	0.710
HOMA-IR(%)	5.9±8.2	2.6±1.2	1.852	0.071
BMI(Kg/m^2)	23.8±1.6	22.9±2.0	1.573	0.123

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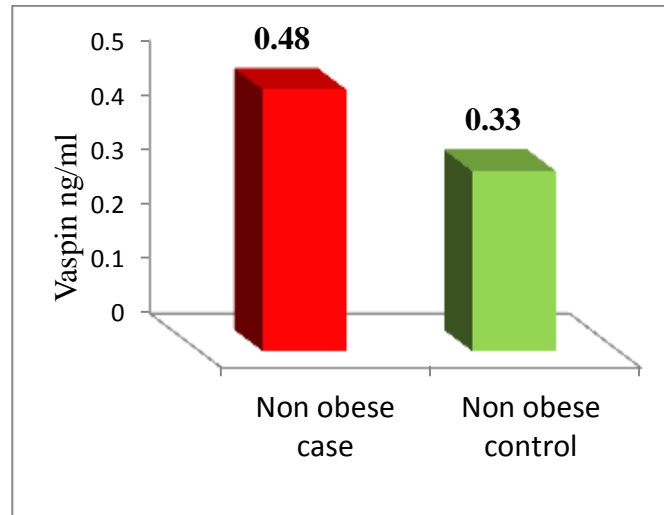
Figure (4.2): Seurm vaspin level in non obese cases and non obese controls.

4.10.2 Cholesterol, triglycerides, HDL-C and LDL-C.

Table 4.11 compares serum lipid profile including cholesterol, triglycerides, HDL-C and LDL-C between non obese cases and non obese controls. The mean levels of triglycerides was found to be significantly higher in non obese cases compared to non obese controls (146.4±55.*4 versus* 106.4±45.6 mg/dl, t=2.613and P=0.012). Cholesterol, HDL-C and LDL-C showed no significant difference in non obese cases compared to non obese controls (P>0.05).

Table (4.11): Comparison between Cholesterol, triglycerides, HDL-C and LDL-C non-obese cases and non-obese controls

Parameter	Non Obese Case (n=22)	Non Obese Control (n=22)	t	P-value
	Mean ± SD			
Cholesterol(mg/dl)	213.3±45.4	199.9±43.9	0.992	0.327
Triglycerides(mg/dl)	146.4±55.4	106.4±45.6	2.613	0.012
HDL(mg/dl)	46.7±9.7	53.2±15.5	1.665	0.105
LDL(mg/dl)	136.8±42.0	124.7±37.6	1.010	0.318

4.10.3 liver and kidney function.

As indicated in table 4.12, there were no significant differences in ALT and AST activities as well as in urea and creatinine concentrations between non obese cases and non-obese controls (P>0.05).

Table (4.12): Comparison between liver and kidney function in non-obese type 2 diabetic patients and non-obese controls**.**

Parameter	Non Obese Case (n=22)	Non Obese Control (n=22)	t	P-value
	Mean ± SD			
AST(U/L)	23.7±11.9	22.7±11.6	0.282	0.779
ALT(U/L)	19.1±7.7	19.6±6.6	0.252	0.802
Urea(mg/dl)	24.6±10.0	20.8±7.9	1.377	0.176
Creatinine(mg/dl)	0.71±0.22	0.63±0.23	1.094	0.280

4.11 Comparison between different studies parameters in obese cases and obese controls.

4.11.1 Vaspin, HbA1c, glucose, insulin, HOMA-IR and BMI

As depicted from table 4.13 and figure 4.3, the mean level of vaspin was significantly increased in obese cases compared to obese controls (0.99±0.7 *versus* 0.62±0.52ng/ml, t=2.036, P=0.048). Similarly, HbA1c, glucose and HOMA-IR were significantly higher in obese cases (6.8±1.6%, 180.9±76.1 mg/dl and 10.1±5.9%*)* than obese controls (4.9±0.68%, 94.4±16.6 mg/dl and 4.6±2.8%, P=0.000, respectively). However, serum insulin increased in obese cases in respect to obese controls with no significant difference (P>0.05).

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Table (4.13): Comparison between studies parameter in obese cases patients and obese controls.

Parameter	Obese Case (n=22)	Obese Control (n=22)	t	P-value
	Mean ± SD			
Vaspin(ng/ml)	0.99±0.7	0.62±0.52	2.036	0.048
HbA1c(%)	6.8±1.6	4.9±0.68	5.366	0.000
Glucose(mg/dl)	180.9±76.1	94.4±16.6	5.206	0.000
Insulin(μIU/ml)	23.8±9.4	18.6±9.7	1.811	0.077
HOMA-IR(%)	10.1±5.9	4.6±2.8	3.985	0.000
BMI(Kg/m^2)	32.9±2.6	33.1±3.9	0.201	0.842
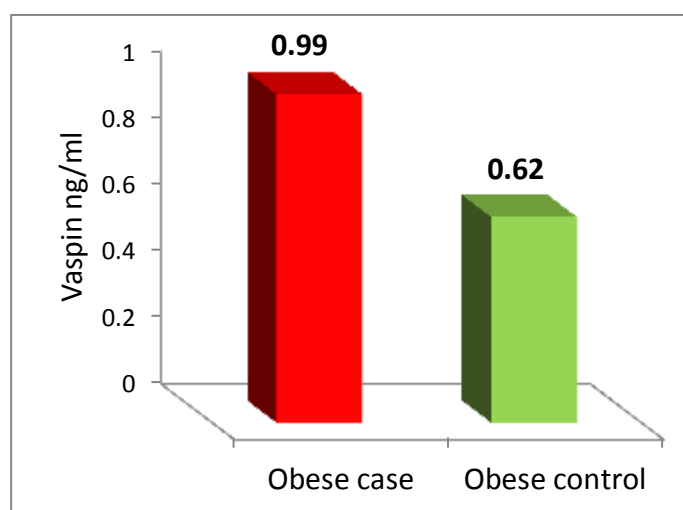
Figure (4.3): Seurm vaspin concentration in obese cases and obese controls.

4.11.2 Cholesterol, triglycerides, HDL-C and LDL-C.

As shown in Table 4.14, the mean levels of cholesterol, triglycerides HDL-C and LDL-C showed no significant differences in obese cases compared to obese controls (P>0.05).

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Table (4.14): Comparison between lipid profile in obese cases and non obese controls

Parameter	Obese Case (n=22)	Obese Control (n=22)	t	P-value
	Mean ± SD			
Cholesterol(mg/dl)	221.8±49.0	219.8±48.2	0.133	0.895
Triglycerides(mg/dl)	228.8±173.3	160.9±83.2	1.657	0.105
HDL-C(mg/dl)	43.9±10.8	48.3±11.7	1.283	0.207
LDL-C(mg/dl)	121.9±69.5	139.3±40.4	1.013	0.318

4.11.3 Comparison between liver and kidney function in obese cases and obese controls.

Table 4.15 indicates that's serum creatinine was significantly increased in obese cases compared to obese controls (0.91±0.31 *versus* 0.64±0.23 mg/dl) with P=0.002. However, no Significant differences were found in ALT and AST activities and urea concentration between obese cases and obese controls (P>0.05).

Table (4.15): Comparison between liver and kidney function in obese cases and obese controls.

| Parameter	Obese Case (n=22)	Obese Control (n=22)	t	P-value
	Mean ± SD			
AST(U/L)	25.1±9.9	25.4±11.0	0.087	0.931
ALT(U/L)	26.3±10.8	20.8±9.01	1.834	0.074
Urea(mg/dl)	25.2±14.7	19.8±6.1	1.595	0.118
Creatinine(mg/dl)	0.91±0.31	0.64±0.23	3.336	0.002

4.12 Comparison between different parameters in obese and non obese cases.

4.12.1 Vaspin, HbA1c, glucose, insulin, HOMA-IR and BMI

As depicted from Table 4.16, BMI was significantly increased in obese cases compared to non obese cases (32.9±2.6 *versus* 23.8±1.6, P=0.000). Vaspin and insulin also significantly increased in obese cases compared to non obese cases (0.99±0.7 ng/ml and 23.8±9.4 µIU/mL *versus* 0.48±0.42 ng/ml and 11.0±9.9

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µIU/mL, P=0.005 and 0.000, respectively. Figure 4.4). However, HbA1c, glucose and HOMA-IR were showed no significant differences (P>0.05).

Table (4.16): Comparison between vaspin, HbA1c, glucose, insulin, HOMA-IR and BMI

Parameter	Obese Case (n=22)	Non Obese case (n=22)	t	P-value
	Mean ± SD			
Vaspin(ng/ml)	0.99±0.7	0.48±0.42	2.950	0.005
HbA1c(%)	6.8±1.6	6.7±0.94	0.373	0.711
Glucose(mg/dl)	180.9±76.1	198.2±82.9	0.724	0.473
Insulin(µIU/ml)	23.8±9.4	11.0±9.9	4.369	0.000
HOMA-IR(%)	10.1±5.9	5.9±8.2	1.958	0.057
BMI(Kg/m^2)	32.9±2.6	23.8±1.6	13.930	0.000

Figure 4.4 seurm vaspin level in obese and non obeses cases
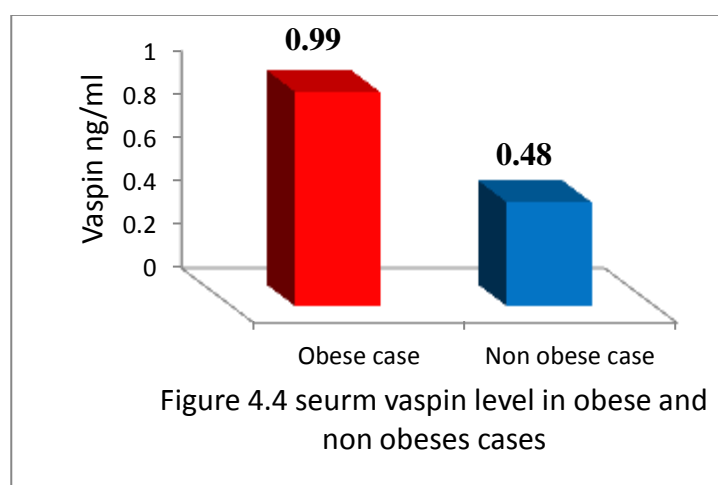
Figure (4.4): Serum vaspin levels in obese and non obese cases.

4.12.2 Cholesterol, triglycerides, HDL-C and LDL-C.

As shown in Table 4.17, triglycerides was significantly increased in obese cases compared to non obese cases (228.8±173.3 versus 146.4±55.4, P=0.039). The mean levels of cholesterol, HDL-C and LDL-C showed no significant differences in obese cases compared to non obese cases (P>0.05).

Table (4.17): Comparison between cholesterols, triglycerides, HDL-C and LDL-C.

Items	Obese Case (n=22)	Non Obese case (n=22)	T	p-value
	Mean ± SD			
Cholesterol(mg/dl)	221.8±49.0	213.3±45.4	0.593	0.556
Triglycerides(mg/dl)	228.8±173.3	146.4±55.4	2.126	0.039
HDL-C(mg/dl)	43.9±10.8	46.7±9.7	0.880	0.384
LDL-C(mg/dl)	121.9±69.5	136.8±42.0	0.861	0.394

4.12.3 liver and kidney function in obese and non obese cases.

There were significant increase in ALT activitiy and creatinine concentrations in obese cases compared to non obese cases (26.3±10.8 U/L and 0.91±0.31 mg/dl *versus* 19.1±7.7 U/L and 0.71±0.22 mg/dl and P=0.015, 0.014). Urea concentration and AST activity showed no significant differences (P>0.05).

Table (4.18): Comparison between liver and kidney function in obese and non-obese cases.

Parameter	Obese Case (n=22)	Non Obese case (n=22)	t	P-value
	Mean ± SD			
AST(U/L)	25.1±9.9	23.7±11.9	0.427	0.671
ALT(U/L)	26.3±10.8	19.1±7.7	2.543	0.015
Urea(mg/dl)	25.2±14.7	24.6±10.0	0.168	0.867
Creatinine(mg/dl)	0.91±0.31	0.71±0.22	2.569	0.014

4.13 Relations of serum vaspin

4.13.1 Seurm vaspin levels in relation to BMI, whole blood HbA1c, serum glucose, insulin levels and HOMA-IR.

Table 4.19 and figure 4.5 gives the relationship of serum vaspin with BMI, whole blood HbA1c, serum glucose, insulin and HOMA-IR. Serum vaspin exhibited a significant positive correlation with BMI (r=0.280 and P=0.008). Similarly Vaspin showed positive significant correlations with blood HbA1c, glucose and HOMA-IR (r=0.209, P=0.049; r=0.290, P=0.006 and r=0.276, P=0.010, respectively). However, the positive correlation between vaspin and insulin was not significant (r=0.145, P=0.178).

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Table (4.19): Serum vaspin level in relation to whole blood HbA1c, serum glucose, insulin levels and HOMA-IR .

Parameter	Vaspin (ng/ml)	
	Pearson correlation (r)	P-value
BMI*(Kg/m^2)	0.280	0.008
HbA1c (%)	0.209	0.049
Glucose (mg/dl)	0.290	0.006
Insulin (µlU/ml)	0.145	0.178
HOMA-IR(%)**	0.276	0.010

*BMI (Kg/m^2): 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 BM≥30.0 were considered obese (WHO, 2012). P<0.05: Significant.
** HOMA-IR Homeostasis Model Assessment Insulin Resistance.
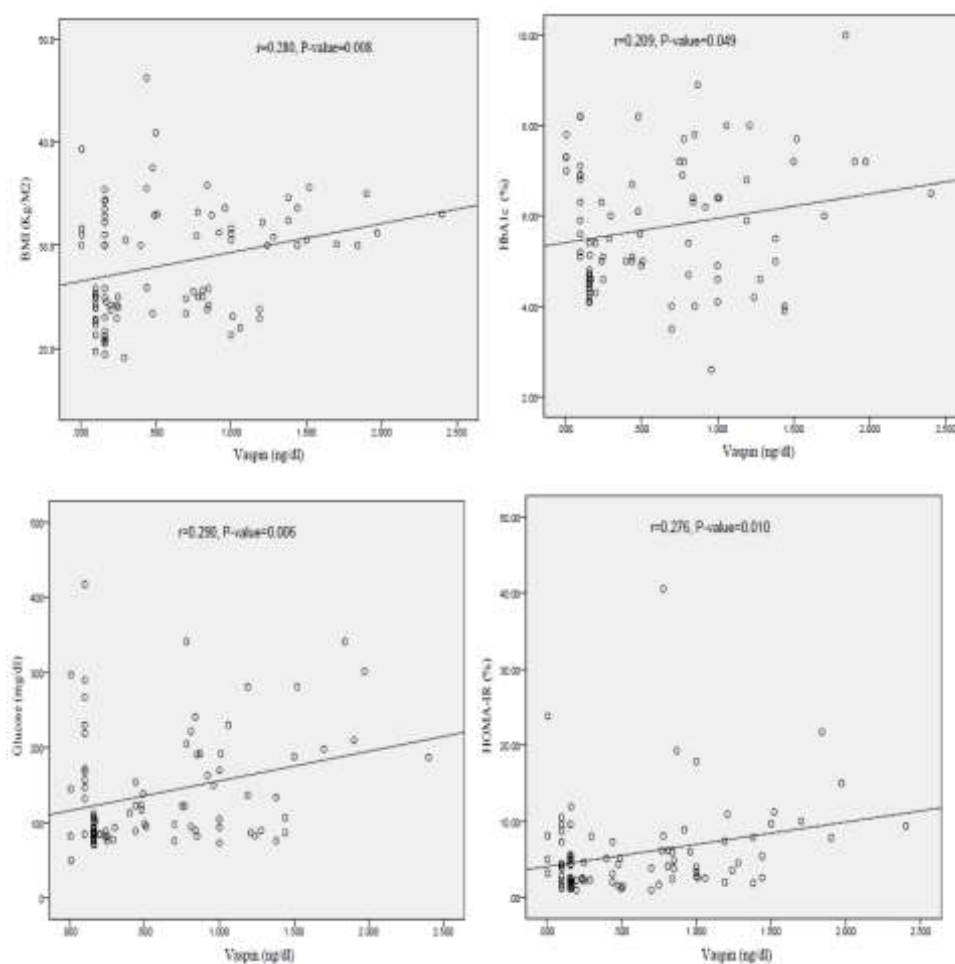
Figure (4.5): Seurm vaspin levels in relation BMI, blood HbA1c, glucose and HOMA-IR.

4.13.2 Seurm vaspin level in relation to serum lipid profile

Serum vaspin level in relation to serum cholesterol, triglycerides, HDL-C and LDL-C summarized in Table 4.20. There was a significant positive correlation between vaspin level and triglycerides (r=0.303, P=0.004, figure 4.5). However, vaspin was not significantly correlated with cholestrol, HDL-C and LDL-C (r=0.060, P=0.578; r=-0.138, P=0.199 and r=-0.122, P=0.258, respectively).

Table (4.20): Serum vaspin level in relation to lipid profile

Parameter	Vaspin (ng/ml)	
	Pearson correlation r	P-value
Cholesterol(mg/dl)	0.060	0.578
Triglycerides(mg/dl)	0.303	0.004
HDL-C *(mg/dl)	-0.138	0.199
LDL-C **(mg/dl)	-0.122	0.258

*HDL-C: High density lipoprotein cholesterol, **LDL-C: Low density lipoprotein cholesterol. P<0.05: Significant, P>0.05: not significant
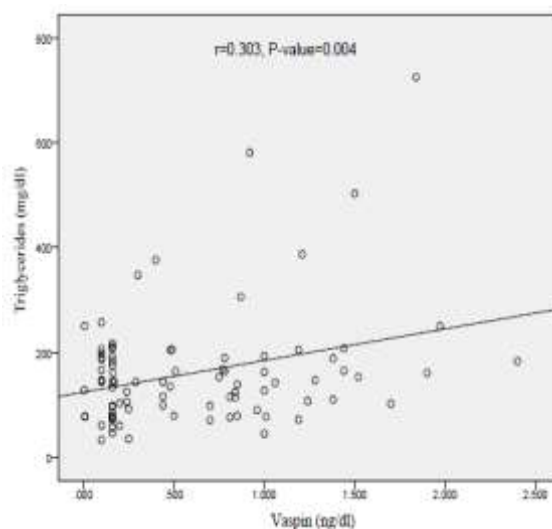
Figure (4.6): Serum vaspin level in relation to triglycerides**.**

4.13.3 Seurm vaspin levels in relation to liver and kidney functions of the study population.

As indicated in Table 4.21 there was a significant positive correlation between vaspin level and ALT activity (r=0.434, P=0.000. Figure 4.6). However no significant positive corrlation were found between vaspin and AST, urea and creatinine (r=0.112, P=0.301; r=0.069, P=0.525 and r=0.192, P=0.073).

Table (4.21) : Serum vaspin levels in relation to liver and kidney function

Parameter	Vaspin (ng/ml)	
	Pearson correlation (r)	P-value
ALT(U/L)	0.434	0.000
AST(U/L)	0.112	0.301
Urea(mg/dl)	0.069	0.525
Creatinine(mg/dl)	0.192	0.073

P<0.05: Significant.
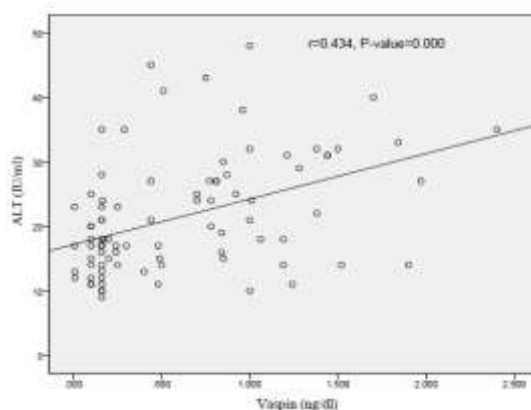
Figure (4.7): Serum vaspin level in relation to ALT activity

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Chapter 5

Discussion

Chapter 5
Discussion

The global prevalence rate of diabetus meliutes is alarming. The total number of people with diabetes worldwide is projected to rise from 285 million in 2010 to 366 million in 2030 (Shaw et al., 2010). Mortality rate in Palestine in 2015 was 2.9 per 1,000 of population, in Gaza Strip 2.7 per 1,000 of population, and in West Bank 3.0 per 1,000 (Moh, 2015), making it one of the most important public health challenges. Despite that, there are under-diagnosis and under-reporting of the disease in the Gaza strip. Biochemical tests of the disease are restricted to monitoring blood glucose level when the patient visits the clinic. This necessitates introducing of other laboratory tests in the course of the disease. Recently, vaspin hormone was linked to type 2 diabetes and obesity (Li et al., 2008; Atya et al., 2013 and Tasnim, Faruque, Hassan & Ali, 2015). In the current study, serum vaspin was estimated in non obese and obese type 2 diabetic patients, in a trial to investigate the role of serum vaspin level in pathogenesis of type 2 diabetes accompanied with obesity.

5.1 Socioeconomic data, physical activity and meal frequency/day of the study population.

The present data indicated that higher number of cases have family history of diabetes than controls. This means that type 2 diabetes is associated with family history. Such finding coincides with that obtained by Annis, Caulder & Cook. (2005); Pijl, Henneman & Claassen. (2009); Abu Mustafa (2011) and Wagner et al. (2013), who reported that family history is a risk factor for type 2 diabetes. On the other hand, employment, family income/month and smoking were not linked with type 2 diabetes. Similarly physical activity and meal frequency/ day was not associated with type 2 diabetes.

5.2 Diabetes duration and self-reported complications of the study population.

The finding that almost half of patients had diabetes since 5 years or less 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 i.e. there is a latent phase before diagnosis of type 2 diabetes. During this period of undiagnosed disease, risk factors for diabetic micro- and macrovascular complications are elevated and diabetic complications are developing (Watkins, 2003). In the present study, self-reported symptoms including retinopathy, cardiovascular disease and neuropathy were evident among diabetic patients. Several studies reported similar diabetic complications with increasing rates upon disease progress (Abu Snayma, 2012 and Hamad, 2013).

5.3 Serum vaspin, HbA1c, glucose and HMOA-IR of the study population.

The current study showed that the mean level of serum vaspin in type 2 diabetic patients was significantly higher than that of the controls. Similarly, HbA1c, glucose and HMOA-IR showed significant increased in type 2 diabetic patients. In comparison, obese and non obese diabetic patients showed increased level of serum vaspin level than their counterparts of obese and non obese controls. The elevation of vaspin level in diabetic patients was previously reported (Li et al., 2012; Atya et al., 2013 and EL said, Sedik & Mohamed., 2014). Interestingly, serum vaspin level in obese diabetic patients was significantly higher than non obese diabetic patients. This implies that obesity contributed to the elevation of vaspin level in diabetic patients. Increased levels of serum vaspin was documented in obesity (Youn et al., 2008; Lee et al., 2008 and Atya et al., 2013). This was supported our finding that serum vaspin was positively correlated with BMI. Such positive association was reported by EL said et al (2014). In addition, Youn et al. (2008) and Esaki et al. (2009) observed a significant BMI –adjusted correlation with vaspin. Finally, It was postulated that the secretion of vaspin from human adipose tissue could represent a compensatory mechanism associated with obesity, severe insulin resistance, and type 2 diabetes

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(Domaa, El-Bagori & El sayd, 2012; El Said et al., 2014 and Dimova & Tankova, 2015).

As indicated in the present study, the mean blood HbA1c and serum glucose level were significantly higher in cases compared to controls. Similar results were obtained (Nicholas, Charlton, Dregan & Gulliford, 2013; Shubrook, 2014 and Tasnim et al., 2015). Excessive production of the early glycation products is an acute reversible change induced by hyperglycemia. These glycation products are formed both inside and outside the cells, as glucose rapidly attaches to the amino groups of the proteins through the non enzymatic process of nucleophilic addition, to form schiff base adducts. Within hours, these adducts reach equilibrium levels that are proportional to the blood glucose concentration and subsequently undergoes rearrangement to form more stable early glycation products, which reach equilibrium over a period of several weeks. One of the protein glycated in this way is glycated haemoglobin (Sultanpur, deepa & kumar, 2010). Once a hemoglobin molecule gets glycated, a buildup of glycated hemoglobin within the red cell therefore reflects the average level of glucose to which the cell has been exposed during its life cycle. Measuring glycated hemoglobin can assesses the effectiveness of therapy by monitoring long-term serum glucose regulation (ADA, 2006).

When related to serum vaspin, blood HbA1c and serum glucose showed positive significant correlations with vaspin. This result is in agreement with Kempf et al. (2010) and El-Mesallamy et al. (2011) who found that serum vaspin levels were significantly correlated with markers of insulin resistance and glycemic indices such as glucose and HbA1c %. Kukla et al. (2010) and Lee et al. (2008) found that vaspin correlated positively with fasting glucose suggesting that vaspin level may play a role in glucose metabolism and may be a part of protective mechanisms aimed to reduce insulin resistance in humans. Recombinant vaspin has been shown to improve glucose tolerance and insulin sensitivity (Hida et al., 2005). In this context, we hypothesize that increased vaspin secretion might represent a compensatory mechanism in response to decrease insulin sensitivity or impairments of glucose metabolism.

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5.4 Serum lipid profile of the study population

The present results demonstrated significant increase in triglycerides and decrease in HDL-C level in cases compared to control. Such findings are in line with that obtained by Yassin et al. (2011) and Atya et al. (2013). The general increase levels of serum lipids in diabetic patients may be mainly attributed to increase in the mobilization of free fatty acids from fat depots. Then, excess fatty acids in serum are converted into triglycerides, phospholipids and cholesterol in liver (Scheen, 2003 and Robciuc et al., 2013). When related to vaspin, triglycerides was positively correlated with serum vaspin. This results agreed with El-Mesallamy et al. (2011) who demonstrated that vaspin levels are significantly correlated with markers of lipid metabolism such as triglycerides, cholesterol, and to a lesser extent LDL-C, indicating that vaspin may play a role in lipid metabolism or might be induced by diabetic dyslipidemia as a compensatory mechanism, especially because vaspin is an adipokine secreted by adipocytes.

5.5 Liver and kidney function of the study population.

In the present study, liver function was assessed through determination of AST and ALT. There were marked elevations in ALT activities in obese cases compared to non obese cases. Such finding are in agreement with the previous studies (Yassin et al. 2011). ALT activity was significantly elevated in obese cases compared with non obese cases, this could be due to direct hepatotoxic effect of fatty acid on the liver when it is produced in excess as observed in the present study. Mechanisms for this may include cell membrane disruption at high concentration, mitochondrial dysfunction, toxin formation, and activation and inhibition of key steps in the regulation of metabolism (Atiba et al., 2013).

In the present study, kidney function was assessed through determination of urea and creatinine. There were significant increases in urea and creatinine concentrations in cases compared to controls. Similar results were obtained (Sharma et al., 2011 and Kanwar et al., 2015). Urea is formed by the liver as an end product of protein breakdown and is one marker of the kidney function (Debra Manzella, 2008 and Sharma et al., 2011). Increase in serum urea observed here may be due to impairment in its synthesis as a result of impaired hepatic function and/or due to

disturbance in protein metabolism. Creatinine is a waste product that is normally filtered from the blood and excreted with the urine. Higher creatinine levels in diabetic patients may be related to disturbance of kidney function (Debra Manzella, 2008,). In addition, the observed elevation in urea and creatinine may be explained on the basis of impairment in glomerular filtration as a result of impairment in kidney function accompanied diabetes mellitus.

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Chapter 6
Conclusion & Recommendation

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6.1 Conclusion

1. Type 2 diabetes mellitus was more frequent among individuals with family history of the disease.

2. The main self-reported complications among diabetic patients were retinopathy, cardiovascular disease and neuropathy.

3. The mean level of serum vaspin was significantly higher in cases compared to controls. Obese diabetic patients showed significantly higher serum vaspin level than non obese patients .

4. The levels of blood HbA1c and serum glucose were significantly increased in cases compared to controls

5. The mean levels of triglycerides was significantly higher in cases compared to controls whereas HDL-C was significantly lower in cases.

6. The concentration of urea and creatinine were significantly higher in cases compared to controls.

7. Serum vaspin levels showed significant positive correlations with BMI, HbA1c, glucose, HOMA-IR, triglycerides and ALT.

6.2 Recommendations

1. Introducing of vaspin test for type 2 diabetic patients in Gaza hospitals is highly recommended.

2. Frequent monitoring of serum vaspin levels as predisposing factor in prediabetics.

3. Regular visits to optical, cardiac and neurological clinics to take early steps to avoid and manage diabetic complications concerning diabetic retinopathy, neuropathy, nephropathy and cardiovascular disease.

4. Further research is needed to asses vaspin role in metabolic syndrome and obesity

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7. Annexes

The Palestinian National Authority	السلطة الوطنية الفلسطينية
Ministry of Health	وزارة الصحة
Directorate General of Human Resources Development	الإدارة العامة لتنمية القوى البشرية

التاريخ: 2016/01/05م

الرقم: ....

الأخ / د. فؤاد العيسوي،،، المحترم،،،

وكيل الوزارة المساعد

السلام عليكم ورحمة الله وبركاته،،،

الموضوع/ تسهيل مهمة باحثة

بخصوص الموضوع أعلاه، يرجى تسهيل مهمة الباحثة/ نجوى طلال اليورنو الملتحقة ببرنامج ماجستير العلوم الحياتية تخصص - تحاليل طبية - الجامعة الإسلامية بغزة في إجراء بحث بعنوان :-
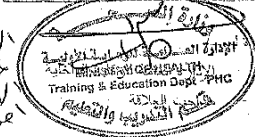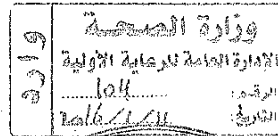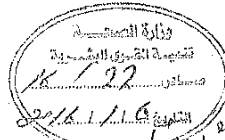
"Serum Vaspin Level in Type2 Diabetic Patients from Gaza Strip"

حيث الباحثة بحاجة لتعبئة استبانه وقياسات الطول والوزن وجزء من عينة دم سحبت لأغراض شخصية من عدد من مرضى السكر النوع الثاني وعينة ضابطة ممن لا يعانون مرض السكر من المراجعين لمراكز الرعاية الأولية في قطاع غزة.

يرجى ترحيبكم لذوي الاختصاص بضرورة الحصول على الموافقة المستنيرة ممن هم على استعداد للمشاركة في البحث ومن ثم تمكين الباحثة من التواصل معهم، وفق الأسس التي يتم بها التعامل مع هذا النوع من العينات ويشرف العاملين في أقسام المختبرات، وبما لا يتعارض مع مصلحة العمل وضمن أخلاقيات البحث العلمي، ودون تحمل الوزارة أي أعباء أو مسئولية.

وتفضلوا بقبول التحية والتقدير،،،

د. ناصر رأفت أبو شعبان
مدير عام تنمية القوى البشرية

Annex 2

Case control study, questionnaire for assessment of Serum vaspin level in type 2 diabetic patients from Gaza strip .

أخي المواطن الكريم/ أرجو مساعدتنا في إتمام هذه الدراسة (بحث ماجستير تحاليل طبية / الجامعة الإسلامية) والتي تختص بمرضى النوع الثاني من السكر, حيث أن هدفنا الوقوف على مسبباته، و خاصة علاقته بالفازبين وذلك للحد من مضاعفاته.

Patients and controls Questionnaire

1. Personal profile of the study population:

Name:_____   Serial No:_____

Age:_____   Tel. No : _____

Gender:_____-

Education   □University or diploma   □ Secondary school

                 □Preparatory school   □Primary school   □Illiterate

2. Socioeconomic data of the study population:

- Employment: □Yes   □No

- Family income: <1000 Shekels …….1000-2000 Shekels ……
                   >2000 Shekels …….

- Family history for Type2 diabetes   □Yes □No
- Smoking: □Yes □No
- Physical activity: □Yes □No
- Meal frequency per day   □One □Two □Three□ four and more

- Duration of Type2Diabetes mellitus/years: **….(only for patients)** _____ year

    4. **Self-reported complications:**

    Retinopathy   □Yes   □No

    Cardiovascular diseases   □Yes   □No

    Neuropathy   □Yes   □No

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4. Anthropometric measurement:

Height (cm): _____   Weight (kg):_____

Body Mass Index: _____

Agreement:

I agree to complete this questionnaire concerning my health statement.

أنا موافق/ ة على تعبئة هذا الاستبيان الذي يتعلق بصحتي.

التوقيع: ....

التاريخ: ....

شكرا لكم على حسن تعاونكم

الباحثة/نجوى طلال البورنو

Annex3

Helsinki Committee
For Ethical Approval

Date: 03/10/2016   Number: PHRC/HC/174/16

الاسم:

Name: NAJWA T. ALBORNO

We would like to inform you that the committee had discussed the proposal of your study about:

نفيدكم علماً بأن اللجنة قد ناقشت مقترح دراستكم

حول:

Serum vaspin level in Type 2 diabetic patients from Gaza strip

The committee has decided to approve the above mentioned research. Approval number PHRC/HC/174/16 in its meeting on 03/10/2016

و قد قررت الموافقة على البحث المذكور عاليه بالرقم والتاريخ المذكوران عاليه

Signature

Member   Member

Chairman

Genral Conditions:-   Speclfic Conditions:-

1. Valid for 2 years from the date of approval.
2. It is necessary to notify the committee of any change in the approved study protocol.
3. The committee appreciates receiving a copy of your final research when completed.

E-Mail:pal.phrc@gmail.com

Gaza - Palestine