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# Assessment of *Helicobacter pylori* infection as risk factor for type 2 diabetes mellitus in Gaza strip

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**By**

**Nabil M. Saadallah**

**Supervisor**

**Prof. Dr. Maged M. Yassin**

**Professor of Physiology**

**Faculty of Medicine**

**The Islamic University of Gaza**

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# *DEDICATION*

***I dedicate this work to:***

***My beloved parents who have always supporting me***

***My brothers and sisters, without their patience, understanding, support and most of all love, this work would not have been possible.***

# *ACKNOWLEDGEMENT*

*All praise is to Allah who has enabled me to complete this thesis.*

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*Last but not least, I offer my regards and blessings to all of those who supported me in any respect during the completion of this work.*

# Assessment of *Helicobacter pylori* infection as a risk factor for type 2 diabetes mellitus in Gaza strip

## Abstract

**Background:** Diabetes mellitus is a multifactorial disorder characterized by disturbance in carbohydrates, lipids and proteins metabolism. It constitutes one of the tenth leading causes of death in Gaza strip with mortality rate of 8.5 per 100,000 population in the year 2010. *Helicobacter pylori* (*H. pylori)* infection is believed to be associated with Type 2 diabetes mellitus.

**Objective:** To assess the *H. pylori* infection as a risk factor for type 2 diabetes mellitus in Gaza strip.

**Materials and methods:** This case-control study comprised 90 type 2 diabetic patients (Cases: 45 males and 45 females) and 90 healthy controls (45 males and 45 females). Questionnaire interview was applied. Blood samples were collected, processed and analyzed. Serum *H. pylori* IgG, glucose, insulin, cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine were determined. Blood glycated hemoglobin (HbA1c) was measured. White blood cell (WBC), red blood cell (RBC), hemoglobin (Hb) and platelet (PLT) were determined. Data were analyzed using SPSS version 18.0.

**Results:** Type 2 diabetes was more prevalent among families with low income as well as among individuals with family history of the disease. More than half of the cases had diabetes since less than 5 years and most of them followed diet. The main self-reported complications were retinopathy, neuropathy and cardiovascular diseases. In addition, the prevalence of gastritis and peptic ulcer was significantly higher among cases compared to controls.

Blood HbA1c and serum glucose and insulin levels was significantly higher in cases compared to controls (8.2±1.7%, 153.7±53.0 mg/dl and 11.6±9.6 μlU/ml vs 5.2±0.7%, 87.0±12.3 mg/dl and 6.8±5.1 μlU/ml, respectively, P=0.000). Serum cholesterol and triglycerides were significantly higher in cases (201.4±43.3 and 203.8±97.7 mg/dl) than controls (189.0±37.9 and 153.1±45.7 mg/dl, P=0.042 and P=0.000, respectively). Serum AST and ALT activities were significantly higher in cases compared to controls (36.3±4.7 and 42.4±5.0 U/L vs 16.9±6.0 and 17.8±8.7 U/L, respectively, P=0.000). Serum urea and creatinine were also found to be significantly higher in cases (47.0±5.3 and 1.06±0.21 mg/dl vs 31.5±11.8 and 0.88±0.22, mg/dl, respectively, P=0.000). White blood cell and PLT counts were significantly increased in cases compared to controls (8.0±1.9 and 262.3±61.3×$10^{9}$/L vs 7.0±1.4 and 224.8±43.4×$10^{9}$/L, respectively, P=0.000) whereas RBC count and Hb content did not show significant differences between cases and controls. The prevalence of *H. pylori* among diabetic patients 65 (72.2%) was significantly higher than controls 33 (36.7%) with P=0.000. Infection with *H. pylori* was significantly higher in diabetic males than diabetic females (P=0.034). When related to *H. pylori*, blood HbA1c levels were significantly higher in positive than in negative cases (8.4±1.8 vs 7.6±1.5, P=0.042). Serum cholesterol, triglycerides and LDL-C levels were significantly increased in *H. pylori* positive cases than in negative cases (216.4±42.5, 190.1±91.9 and 139.8±42.6 mg/dl, vs 195.6±42.6, 164.5±61.2 and 115.4±40.2 mg/dl, P=0.041, P=0.033 and P=0.013, respectivily), whereas HDL-C level was significantly lower in positive cases (37.5±6.9 vs 41.1±8.8 mg/dl, P=0.040). The activity of serum ALT and the concentration of urea were significantly increased in *H. pylori* positive cases compared to negative cases (43.1±4.9 U/L and 41.1±10.9 mg/dl vs 40.8±4.8 U/L and 37.0±12.8 mg/dl, P=0.049 and P=0.022, respectivily). The WBC count was also singnificantly elvated in *H. pylori* positive cases (8.1±1.8 vs 7.2±1.5 ×$10^{9}$/L, P=0.038).

**Conclusions:** *H. pylori* infection was significantly higher in type 2 diabetic patients compared to controls. *H. pylori* infection was associated with blood HbA1c, serum cholesterol, triglycerides, LDL-C, HDL-C, ALT, urea and WBC count. Therefore, monitoring of *H. pylori* infection as a possible risk factor of type 2 diabetes may be of prognostic value.

**Keywords:** *Helicobacter pylori*, Type 2 diabetes, Gaza Strip.

# تقييم الإصابة بالجرثومة الملوية البوابية كعامل اختطار لمرضى السكري النمط الثاني في قطاع غزة

## ملخص الدراسة


**مقدمة**: مرضى السكري هو خلل وظيفي متعدد العوامل، يتصف بحدوث اضطراب في العمليات الأيضية لكل من الكربوهيدرات، الدهون والبروتينات، و يعد هذا الخلل أحد الأسباب العشرة المؤدية للوفاة في قطاع غزة بمعدل ٨,٥ في كل ١٠٠.٠٠٠ شخص من التعداد السكاني حسب إحصائية سنة ٢٠١٠، كما يعتقد بأن الإصابة بالجرثومة الملوية البوابية مرتبطة بسكري النمط الثاني.

**الهدف:** تهدف الدراسة إلى تقييم الإصابة بالجرثومة الملوية البوابية كعامل اختطار لمرضى لسكري النمط الثاني في قطاع غزة.

**الطرق والأدوات:** اشتملت هذه الدراسة المشهدة على ٩٠ حالة مرضية من مرضى سكري النمط الثاني (٤٥ ذكور، ٤٥ إناث) و٩٠ شخصا من الأصحاء كعينات ضابطة، وقد تم عمل مقابلات تم فيها تعبئة الاستبيانات، كذلك جمعت عينات الدم ثم تم معالجتاها وتحليلها، كما تم قياس كل من الجلوبيولين المناعي ج للجرثومة الملوية البوابية، الجلوكوز، الأنسولين، الكولسترول، الدهون الثلاثية، كلسترول البروتين الشحمي خفيض الكثافة، كلسترول البروتين الشحمي مرتفع الكثافة،الإنزيم الناقل للأسبارتات أمين،الإنزيم الناقل للألانين أمين، اليوريا والكرياتينين، بالإضافة لذلك تم قياس الهيموجلوبين السكري A1c. كذلك تم قياس كل من خلايا الدم البيضاء، خلايا الدم الحمراء، الهيموجلوبين والصفائح الدموية.

استخدم البرنامج الإحصائي SPSS-18.0 لتحليل البيانات والنتائج.

**النتائج:** أظهرت النتائج بأن سكري النمط الثاني يعتبر أكثر انتشارا بين العائلات الأقل دخلا بالإضافة إلى الأشخاص ذوي التاريخ العائلي للمرض، كما أظهرت النتائج بأن أكثر من نصف الحالات المرضية كانوا حاملين لمرض السكري منذ أن كانت أعمارهم أقل من ٥ سنوات وأغلب هذه الحالات يتبعون حمية غذائية. كما أظهرت الدراسة بأن التعقيدات الناتجة عن المرض هي اعتلال الشبكية، اعتلال الكلية وأمراض القلب والأوعية الدموية. كما أظهرت النتائج وجود ارتفاع في انتشار التهاب المعدة والقرحة الهضمية عند الحالات المرضية مقارنة بالأصحاء، وهذه النتيجة ذات دلالة إحصائية. وأوضحت النتائج وجود زيادة ذات دلالة إحصائية في مستويات كل من الهيموجلوبين السكري A1c، الجلوكوز والأنسولين عند الحالات المرضية مقارنة بالأصحاء،

كما أشارت النتائج إلى أن كل من الكلسترول والدهون الثلاثية قد ازدادت في الحالات المرضية مقارنة بالأصحاء، وهذه الزيادة ذات دلالة إحصائية، كما بينت النتائج وجود زيادة ذات دلالة إحصائية في نشاط كل من الإنزيم الناقل للأسبارتات أمين والإنزيم الناقل للألانين أمين في الحالات المرضية مقارنة بالأصحاء، بالإضافة إلى ذلك وجدت زيادة ذات دلالة إحصائية في مستويات اليوريا والكرياتينين في الحالات المرضية، كما وجد ارتفاع ذو دلالة إحصائية في أعداد كل من كرات الدم البيضاء والصفائح الدموية عند الحالات المرضية مقارنة بالأصحاء، بينما لم تظهر النتائج فروقات ذات دلالة إحصائية في أعداد كرات الدم الحمراء ومحتوى الهيموجلوبين بين الحالات المرضية والأصحاء.

وأظهرت النتائج زيادة في انتشار الجرثومة الملوية البوابية بين مرضى السكري مقارنة بالأصحاء، كما أشارت النتائج إلى وجود زيادة في الإصابة بالجرثومة الملوية البوابية عند مرضى السكري الذكور بالمقارنة مع مرضى السكري الإناث. وعند عمل علاقة مع الجرثومة الملوية البوابية نجد بأن مستويات الهيموجلوبين السكري A1c هي أعلى عند الحالات المرضية المصحوبة بالجرثومة الملوية البوابية من تلك الحالات المرضية الغير مصحوبة بالجرثومة الملوية البوابية، كما أظهرت النتائج وجود زيادة في مستويات كل من الكلسترول، الدهون الثلاثية، وكلسترول البروتين الشحمي خفيض الكثافة عند الحالات المرضية المصحوبة بالجرثومة الملوية البوابية أكثر من الحالات المرضية الغير مصحوبة بالجرثومة الملوية البوابية، وهذه النتيجة ذات دلالة إحصائية، بينما وجد انخفاض ذو دلالة إحصائية في مستوى كلسترول البروتين الشحمي مرتفع الكثافة عند الحالات المرضية المصحوبة بالجرثومة. وأشارت النتائج لوجود زيادة في كل من نشاط الإنزيم الناقل للأسبارتات أمين و تركيز اليوريا عند الحالات المرضية المصحوبة بالجرثومة الملوية البوابية مقارنة مع الحالات المرضية الغير مصحوبة بالجرثومة الملوية البوابية، وهذه الزيادة ذات دلالة إحصائية. كذلك وجد ارتفاع ذو دلالة إحصائية في عدد كرات الدم البيضاء عند الحالات المرضية المصحوبة بالجرثومة الملوية البوابية.

**الاستنتاجات:** أشارت النتائج بأن معدل الإصابة بالجرثومة الملوية البوابية أعلى عند مرضى سكري النمط الثاني بالمقارنة مع الأصحاء، وهذه النتيجة ذات دلالة إحصائية، وأظهرت النتائج بأن الإصابة بالجرثومة الملوية البوابية مقترنة بكل من الهيموجلوبين السكري A1c، الكولسترول، الدهون الثلاثية، كلسترول البروتين الشحمي خفيض الكثافة، كلسترول البروتين الشحمي مرتفع الكثافة، الإنزيم الناقل للألانين أمين اليوريا وعدد كرات الدم البيضاء، لذلك فإن التحكم بالإصابة بالجرثومة الملوية البوابية كعامل اختطار محتمل لسكري النمط الثاني له قيمة سريريه.

**الكلمات المفتاحية:** الجرثومة الملوية البوابية، سكري النمط الثاني، قطاع غزة.

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# List of abbreviations

| Abbreviation | Full name |
|---|---|
| ADA | American diabetes association |
| AGEs | Advanced glycosylated end-products |
| ALT | Alanine aminotransferase |
| AST | Aspartate aminotransferase |
| BMI | Body mass index |
| CagA | Cytotoxin associated gene A |
| CBC | Complete blood count |
| CRP | C- Reactive protein |
| CVD | Cardiovascular disease |
| DNA | Deoxyribonucleic acid |
| EDTA | Ethylene diamine tetra acetic acid |
| ELISA | Enzyme linked immunosorbent assay |
| GIT | Glucose dependent insulin tropic peptide |
| GLP-1 | Glucagon-like peptide-1 |
| *H. pylori* | *Helicobacter pylori* |
| HbA0 | Non Glycosylated hemoglobin |
| HbA1C | Glycated hemoglobin |
| HDL-C | High density lipoprotein cholesterol |
| HMG-CoA | Hydroxyl methy glutary-CoA |
| HpSA | *Helicobacter pylori* stool antigen |
| IDF | International diabetes federation |
| IgA | Immunoglobulin A |
| IgG | Immunoglobulin G |
| IMX | Immunoassay |
| LDL-C | Low density lipoprotein cholesterol |
| MALT | Mucosa associated lymphoid tissue |
| MEIA | microparticle enzyme immunoassay |
| MEIA | Microparticle enzyme immunoassay |
| MOH | Ministry of health |

| | |
|---|---|
| PCR | Polymerase chain reaction |
| PLT | Platelets |
| RBC | Red blood cell |
| RUT | Rapid urease test |
| SPSS | Statistical package for social sciences |
| TG | Triglycerides |
| TNF | Tumor necrosis factor- α |
| UBT | Urea breath test |
| USA | United states of america |
| WBC | White blood cell |
| WHO | World health organization |

# Chapter 1

# Introduction

## 1.1 Overview

Diabetes mellitus is a 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 mellitus 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 et al., 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 et al., 2000; Guyton and Hall, 2006 and Holt and 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 et al., 2007)**. In addition, disturbance in liver and kidney functions was also reported in type 2 diabetes **(Sharma et al., 2011; Yassin et al., 2011 and Atiba et al., 2013)**.

*Helicobacter pylori* (*H. pylori*), is a gram negative spiral shaped bacterium that is found in the gastric mucous layer or adherent to the epithelial lining of the stomach. The presence of *H. pylori* confers a six fold increased risk of gastric adenocarcinoma, account for half of all gastric cancers and strongly implicated in the development of gastric B cell mucosa associated lymphoid tissue (MALT) lymphomas as well as it causes peptic ulcer disease **(Morgner et al., 2000; Lehours and Yilmaz, 2007; Mehmood et al., 2010 and Kate et al., 2013)**.

Recent reports suggested that *H. pylori* might have high prevalence among patients with diabetes. An increased prevalence of *H. pylori* infection among diabetes mellitus patients was first suggested by a report from Hungary **(Simon et al., 1989; Devrajani et al., 2010 and Taher et al., 2012)**. It was further supported by other studies from developing and under developed countries **(Oldenburger et al., 1996; Gentile et al., 1998; Devrajani et al., 2010 and Jeon et al., 2012)**. The latter study from Iran documented a *H. pylori* prevalence rate of 74.4% in type 2 diabetes mellitus patients as against 50% in non-diabetic controls **(Taher et al., 2012)**. In Gaza strip, only one study focused on *H. pylori* infection and malnutrition among type 2 diabetic medical services patients **(Abu Jabal, 2012)**. The present study is the first to assess *H. pylori* infection in type 2 diabetic patients and its relation to biochemical and hematological parameters.

## 1.2 General objective

To assess the *H. pylori* infection as a risk factor for type 2 diabetes mellitus in Gaza strip.

## 1.3 Specific objectives

1. To determine the prevalence of *H. pylori* infection among the cases of diabetic patients compared with controls.
2. To assess the level of glycated hemoglobin (HbA1c) in blood and serum insulin and glucose in cases and controls.
3. To measure lipid profile include cholesterol, triglycerides, LDL-C and HDL-C) in the cases and controls.
4. To estimate liver function through determination of AST and ALT as well as kidney function through determination of urea and creatinine.
5. To evaluate blood parameter including WBC, RBC, hemoglobin and PLT in cases compared to controls.
6. To verify the relationship between *H. pylori* and the studied parameters in diabetic patients.

## 1.4 Significance

1. Type 2 diabetes mellitus becomes one of the leading cause of death globally as well as in Palestine according to Ministry of Health (MOH) report **(Ministry of Health, MOH, 2010)**.
2. To find out whether exposure to *H. pylori* infection is associated with type 2 diabetes mellitus, may be of prognostic value.
3. Only one previous to investigated the role of *H. pylori* in malnutrition among diabetic patients in Gaza strip. This will be the first study to assess *H. pylori* infection in type 2 diabetic patients and its relation to biochemical and hematological parameters.
4. Understanding the role of *H. pylori* in diabetes mellitus could be useful in the management of the disease.

# 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 mellitus

This form of diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin dependent diabetes, type 1 diabetes, 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 et al., 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 mellitus 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 et al., 2009)**. Similarly, children of women with gestational diabetes are known to be at risk for obesity and diabetes mellitus in their later life **(Bánhidy et al., 2011)**.

## 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 et al., 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 glucose appearance. After reaching a post-meal peak, blood glucose slowly decreases during the next several hours, eventually returning to fasting levels **(Shrayyef and 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 $\alpha$- and $\beta$-cells, which results in glucagon suppression **(Camacho et al., 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 et al., 2000; Guyton and Hall, 2006 and Holt and 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 et al., 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 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 et al., 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 **(MOH, 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 UN 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)**. However, in Palestine, there is under diagnosis and under reporting of the disease. This is due to lack of proper hospital and clinic recording system **(MOH, 2005)**. 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)**. The mortality rate of diabetes mellitus among Palestinians constituted 5.9 per 100,000 population in the year 2009 **(MOH, 2009)**, and this figure raised to 8.5 per 100,000 population in the year 2010 **(MOH, 2010)**.

## 2.3.3 Risk factors of type 2 diabetes

### 2.3.3.1 Body Mass Index

Many studies have reported that increased BMI is a strong risk factor for type 2 diabetes **(ADA, 2013 and Almdal et al., 2008)**. A strong positive association between obesity and type 2 diabetes is found both in men and women **(Eckel et al., 2011 and Almdal et al., 2008)**. 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 et al., 2006 and Ebe et al., 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 et al., 2009)**.

### 2.3.3.2 Lipids

Unfavorable blood lipids has been reported as a risk factor for type 2 diabetes **(Njolstad et al., 1998; Almdal et al., 2008 and Rutter and nesto 2011)**. An inverse relationship between HDL-C and risk of type 2 diabetes have been documented **(Jacobsen et al., 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 Hypertension

Hypertension have shown as a progression an independent predictor of type 2 diabetes **(Conen et al., 2007 and Movahed et al., 2010)**. Endothelial dysfunction could be one of the common pathophysiological pathways explaining the strong association between blood pressure and incident type 2 diabetes **(Meigs et al., 2006a)**. Markers of inflammation such as C-reactive protein have been consistently related to incident of type 2 diabetes **(Hu et al., 2004)**, and to increasing blood pressure levels **(Blake et al., 2003)**, suggesting that, inflammation might be another explanatory factor for the association between blood pressure, the metabolic syndrome, and incident type 2 diabetes **(Ridker et al., 2003)**. In addition, evidence from cross sectional and cohort studies suggests a strong relation between blood pressure and BMI and risk of type 2 diabetes **(Czernichow et al., 2002 and Meigs et al., 2006b)**.

### 2.3.3.4 Physical inactivity

Recent studies have found that physical inactivity is a strong 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 et al., 2003 and Krishnan et al., 2009)**. 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 et al., 2006 and ADA. 2013)**.

### 2.3.3.5 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 et al., 2009 and Kurotani et al., 2012)**. Higher dietary glycemic index has been consistently associated with elevated risk of type 2 diabetes **(Villegas et al., 2007 and Salvado et al., 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 et al., 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 et al., 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 et al., 2012)**.

### 2.3.3.6 Genetics

Several studies have found that, genetic components plays an important role in pathogenesis of type 2 diabetes, 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 et al., 2007 and Ma et al., 2008, Frank and 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 and 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 **(Lyssenko et al., 2008; Bao et al., 2013 and Lyssenko and 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 non ketotic coma, lactic acidosis and hypoglycemia. The chronic complications include diabetic retinopathy, diabetic neuropathy and cardiovascular disease **(Susztak et al., 2003;Becker, 2009 and Yassin et al., 2011)**.

#### 2.3.4.1 Diabetic retinopathy

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 Diabetic neuropathy

Diabetic neuropathy can affect nerves in many different parts of the body. The most common early symptoms of the condition are numbness, tingling, or sharp pains in the feet or lower legs. An estimated 50% of those with diabetes have some form of neuropathy, but not all with neuropathy have symptoms.

The highest rates of neuropathy are among people who have had the disease for at least 25 years. Diabetic neuropathy also appears to be more common in people who have had problems controlling their blood glucose levels, in those with high levels of blood fat and blood pressure, overweight people, and people over the age of 40. 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 et al., 2006)**.

### 2.3.4.3 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, 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, 2005)**.

## 2.4 *Helicobacter pylori*

### 2.4.1 Definition and general characteristics

*Helicobacter pylori* is a spiral or slightly curved gram negative rod with 2-6 characteristic unipolar flagella (Figure 2.1). The bacterium has bluntly rounded ends and measures 2.5-4.0 µm in length and 0.5-1.0 µm in width. The cell wall is smooth and may be coated with a prominent glycocalyx with a thickness of up to 40 nm **(Goodwin et al., 1989)**. The flagella measure 2.5 µm in length and around 30 nm in thickness, and have a distinctive terminal bulb **(Goodwin & Worsley, 1993)**. The bacterium displays remarkable motility in viscous solutions, and the flagella play a central role in this motility **(Hazell et al., 1986 and Suerbaum et al., 1993)**. *H. pylori* is a microaerophilic and under certain circumstances it can be U- shaped or coccoid **(Enroth et al., 1999)**. It resides naturally in the gastrointestinal tract of humans and animals **(Fox, 2002)**. In the stomach, the majority of *H. pylori* can be found in the gastric mucosa; however a few are found adhered to the gastric mucosal epithelium. The bacterium is highly adapted to survive in the hostile environment of the stomach where few other organisms can survive. Although, *H. pylori* is considered to be an extra cellular bacteria, there is evidence suggesting that the bacteria has a mechanism for intracellular invasion **(Kusters et al., 2006)**.

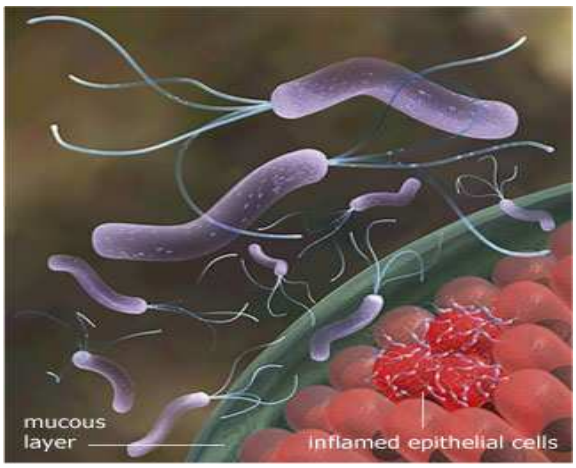



Figure 2.1 *Helicobacter pylori*. The curved bacillus with unipolar flagella is visualized by a scanning electron microscope **(Charles and Janeway, 2005)**.

## 2.4.2 Taxonomy of *Helicobacter pylori*

The scientific classification of the *H. pylori* **(Marshall & Warren, 1984)** is:

**Kingdom**: Bacteria

**Phylum**: Proteobacteria

**Class**: Epsilon Proteobacteria

**Order**: Campylobacterales

**Family**: Helicobacteraceae

**Genus**: Helicobacter

**Species:** *Helicobacter pylori*

## 2.4.3 Prevalence of *Helicobacter pylori* infection

Infection with *H. pylori* has been recognized as a public health problem worldwide affecting approximately 50% of the world population **(Bender et al., 2007 and Sachs and Scott, 2012)**. In developing countries the prevalence of *H. pylori* antibodies was found more than 70% in the populations **(Nurgalieva et al., 2002 and Stasi et al., 2008)**. On the contrary, in developed countries, *H. pylori* infection is less common in young children and increases with age and reaches 50% by adulthood **(Lane et al., 2006 and Zhou et al., 2012)**. In the Gaza strip, Abu-Mughesieb study show that the rate of *H. pylori* infection in Gaza strip was 48.3% **(Abu-Mughesieb, 2007)**. In a recent study focused on *H. pylori* infection and malnutrition, **Abu Jabal (2012)** reported that 70.5% prevalence of *H. pylori* among type 2 diabetic medical services patients in Gaza strip.

## 2.4.4 Transmission of *Helicobacter pylori*

### A) Person-to-person route

Humans are the only known significant reservoir of *H. pylori* **(Collazo, 2012)**. Person to- person contact is believed to be the primary route of transmission in developed countries, and is also important in developing countries. Close personal contact, particularly within the family including mother/parents to child, sibling to sibling and spouse to spouse, has been consistently

demonstrated as a risk factor for transmission of infection **(Escobar and Kawakami, 2004 and Khalifa et al., 2010)**.

### B) Oral-oral route

*Helicobacter pylori* deoxyribonucleic acid (DNA) has been detected in the saliva of *H*. *pylori* positive subjects by polymerase chain reaction (PCR) **(Khalifa et al., 2010 and Collazo, 2012)**. *H. pylori* organisms have also been successfully detected from the dental plaque of infected persons **(Sousa et al., 2006 and Rasmussen et al., 2010)**. In general, isolation has not been uniformly successful, however, perhaps as a result of the transient presence of *H. pylori* in the oral cavity or poor detection capability resulting from the co-occurrence of many other bacteria in the oral cavity.

### C) Fecal-oral route

Fecal- oral is the main route of *H. pylori* transmission, *H. pylori* has been detected in faeces by culture and its DNA by PCR **(Delport et al., 2007; Mishra et al., 2008 and Momtaz et al., 2012)**, although other investigators have failed to replicate this **(Van Zwet et al., 1994)**. These data, together with those from **Silva et al. (2009)**, documented the possible role of fecal shedding of *H. pylori* into the environment.

### D) Iatrogenic transmission

Endoscopes used routinely in upper gastrointestinal procedures may be the source of iatrogenic infection as a result of improper disinfection between procedures **(Brown, 2000)**.

## 2.4.5 Signs and symptoms of *Helicobacter pylori* infection

Most people with *H. pylori* infection are asymptomatic, but a proportion of infected individuals develop severe gastro duodenal diseases, including reflux esophagitis, duodenal ulcer, gastric ulcer, gastric adenocarcinoma and MALT lymphoma **(Peek, 2004; Chen et al., 2013; Shiota et al., 2013 and Witkowska and Smolewski, 2013)**. Acute *H. pylori* infection in adults is accompanied by mild to moderate dyspeptic symptoms and occasional vomiting, which appear few days after challenge, peak during the second

week and then resolve. The clinical course of chronic *H. pylori* infection is highly variable and influenced by microbial, host and environmental factors. In virtually all infected individuals *H. pylori* causes chronic inflammation in the gastric mucosa. Gastritis develops rapidly after acquisition of *H. pylori* infection and persists through several years of the infection, chronic gastritis may gradually progress to atrophic gastritis. (**Oona et al., 2004 and Vale and Vítor, 2010)**.

### 2.4.6 Diagnosis of *Helicobacter pylori* infection

Diagnosis of infection is usually made by checking for dyspeptic symptoms and by tests which can indicate *H. pylori* infection **( Stenström et al., 2008)**. The diagnostic tools for *H. pylori* are serology, rapid urease test (RUT), urea breath test (UBT), endoscopy and biopsy/histopathology, PCR, for DNA of *H. pylori* and *H. pylori* stool antigen (HpSA) **(Tiwari et al., 2005)**. The simplest test of *H. pylori* is serologic, including the assessment of specific IgG level in serum **(Suerbaum et al., 2002)**.

### 2.4.7 Pathogenic mechanisms of *Helicobacter pylori* which predispose to diabetes mellitus

In addition to its association with severe gastrointestinal pathologies **(Nguyen et al., 2010 and Türkay et al., 2011)**, *H. pylori* is associated with other conditions such as atherosclerosis, insulin resistance, diabetes mellitus and some autoimmune diseases **(Manco et al., 2010 and Assal et al., 2013)**. Several hypotheses were presented for confirmation of higher prevalence of *H. pylori* infection in diabetic patients such as immune system impairment, reduction of both gastrointestinal motility and acid secretion and higher secretion of pro-inflammatory cytokines related to the *H. pylori* gastric infection itself **(Bener et al., 2007)**. Inflammation and activated innate immunity have been implicated in pathogenesis of diabetes through insulin resistance, for example, elevated levels of inflammatory cytokines may lead to phosphorylation of serine residues on the insulin receptor substrate, which prevents its interaction with insulin receptors, inhibiting insulin action **(Wellen et al., 2005 and Manco et al., 2010)**. *Helicobacter pylori* specific antigens were detected in the affected islets in a subset of diabetic patients, suggesting

that, bacteria or their slowly degradable remnants may initiate and sustain chronic inflammation in the pancreas **(Miklossy et al., 2008)**.

## 2.5 Related studies

**Oldenburg et al. (1996)** assessed the prevalence of *H. pylori* in diabetes mellitus. A serological test was used to detect antibodies to *H. pylori* in patients. Within six months, 45 type 1, 98 type 2 diabetes, and a control group of 159 outpatients were enrolled in this study. The age adjusted seroprevalence rates of *H. pylori* were determined using a commercial anti-*H. pylori* IgG and IgA enzyme linked immunoassay (ELISA). The prevalence rates increased with age in all age groups until 60–70 years. In diabetic patients, the frequency of *H. pylori* infection was higher than in control subjects in nearly all age groups.

The prevalence of *H. pylori* in type 2 diabetic patients and its relationship with dyspeptic symptoms were evaluated **(Gulcelik et al., 2005)**. Seventy eight type 2 diabetic patients (54 females, 24 males, mean age: 51.9±10.6 year) and 71 non-diabetic control subjects were involved in the study. Patients were questioned for dyspeptic symptoms. Upper gastrointestinal tract endoscopy was performed for all patients and gastric biopsies were obtained and searched for *H. pylori*. The prevalence of *H. pylori* was significantly higher in diabetic patients than in control subjects (75.6 vs 46.0%, $P<0.05$). *H. pylori* infection was found to be correlated with dyspeptic symptoms in diabetic patients.

**Bener et al. (2007)** studied the association between *H. pylori* infection and type 2 diabetes mellitus in the United Arab Emirates population. The study was conducted at the primary health care clinics during the period from June 2002 to August 2003. The study included 210 type 2 diabetic patients and 210 non-diabetic subjects. *H. pylori* was assessed by histopathological examination by measuring antibody profiles (IgG and IgA) among type 2 diabetic patients and the non-diabetic group. A positive antibody titer for *H. pylori* infection (IgA≥300) was found in 76.7% of the diabetic patients compared to 64.8% of the non-diabetic subjects ($P<0.009$). There was higher

prevalence of *H. pylori* infection in diabetic obese patients than the non-diabetic subjects (23.6% vs 11.8%, P<0.001). In addition, **Demir et al. (2008)** found higher prevalence of *H. pylori* infection among type 2 diabetic patients than non diabetic controls.

**Devrajani et al. (2010)** determined the frequency of *H. pylori* infection in diabetic and non-diabetic patients. The study was hospital-based case-control conducted on 148 subjects and divided into two groups: type 2 diabetics and non-diabetics; each group consisting of 74 patients. All diabetic patients of ≥35 years of age, both gender and the known cases with history of dyspepsia, epigastric pain or bloating for more than a month were screened for *H. pylori* infection. Among the diabetic group, HpSA was positive in 54/74 (73.0%), whereas in the non-diabetic group HpSA was positive in 38/74 (51.4%) cases. Fasting blood glucose was identified as low in 4 (5.4%) *H. pylori* infected - diabetic patients.

**Taher et al. (2012)** assessed the prevalence of *H. pylori* infection in diabetes mellitus and studied the relationship between histological findings and *H. pylori* infection in diabetic patients. Eighty patients with dyspepsia that were referred to Gastrointestinal Department between May 2007 and May 2008 were included in the study. Fasting blood sugar for all of the study samples was checked. All of patients underwent upper endoscopy and biopsy specimens were obtained from the antrum and the corpus. The prevalence of *H. pylori* infection was significantly higher in diabetics than in non-diabetics (P=0.001). The prevalence of gastritis did differ significantly between the two groups (P=0.001). The authors concluded that diabetes mellitus is one of the risk factor that must be considered in evaluation of *H. pylori* infection in diabetic patients with dyspepsia. In addition, **Abu Jabal (2012)** reported 70.5% prevalence of *H. pylori* among type 2 diabetic medical services patients in Gaza strip**.**

**Zhou et al. (2013 )** conducted a meta-analysis study to quantify the association between *H. pylori* infection and diabetes. Forty-one studies were identified, involving 14,080 patients, with a total *H. pylori* infection rate of 42.3%. The OR for *H. pylori* infection was increased to 1.33 (95% CI: 1.08-

1.64; P=0.008) among the patients with diabetes. Subgroup analysis revealed a significant higher infection rate of *H. pylori* in the type 2 diabetes group versus the control group: OR=1.76, 95% CI: 1.40-2.21, P<0.00001. The pooled data suggests a trend toward more frequent *H. pylori* infections in diabetes patients, especially in type 2 diabetes patients.

# Chapter 3

# Materials and Methods

## 3.1 Study Design

Case control study design.

## 3.2 Study population

The study population included type 2 diabetic patients (cases) aged 38-62 years attending diabetic clinics at Al-Shifa hospital, Gaza Strip. Controls were apparently healthy non diabetic individuals.

## 3.3 Sampling and sample size

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 Al-Shifa hospital, 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 based on the formula for case-control studies. EPI-INFO statistical package version 3.5.1 (EPI-INFO, 2008) 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 81:81. For a no-response expectation, the sample size was increased to 90 patients. The controls also comprised of 90 healthy individuals.

## 3.4 Exclusion criteria

- Type 1 diabetic patients.
- Gastrointestinal tract related diseases.

## 3.5 Ethical Considerations

An official letter of request sent to MOH to Al-Shifa hospital administration to facilitate the conduction of the study **(Annex 1)**. In addition, the necessary

approval to conduct the study was obtained from Helsinki committee in the Gaza Strip **(Annex 2)**. Helsinki committee is an authorized professional body for giving permission to researchers to conduct their studies with ethical concern in the area.

## 3.6 Data collection

### 3.6.1 Questionnaire interview

A meeting interview was used for filling a questionnaire which designed for matching the study need for both cases and controls **(Annex 3)**. All interviews were conducted face to face by the researcher himself. During the survey the interviewer explained any of the questions that were not clear. The questionnaire was based on the questions of a previous study with some modifications **(Asfeldt et al., 2009 and and Hamam, 2013)**. Most questions were the yes/no questions which offer a dichotomous choice **(Backestrom and Hursh-Cesar, 2012)**. The validity of the questionnaire was tested by six specialists in the fields of Microbiology, Endocrinology, Epidemiology and Public Health. The questionnaire was piloted with 10 patients not included in the study. The questionnaire included questions on the personal profile of the study population (Age, gender and education), socioeconomic data (employment, family income, family history of diabetes and smoking), physical activity, diet and compliance of medication, duration of diabetes, self-reported complications (cardiovascular disease, retinopathy and neuropathy), gastritis and peptic ulcer among the study population.

### 3.6.2 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 shoses 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)**.

### 3.6.3 Specimen collection and biochemical analysis

Twelve hours fasting overnight venous blood samples were collected from 90 type 2 diabetic patients and 90 healthy non diabetic controls. Blood samples (6 ml each) were drawn by a well trained nurse into vacutainer and plastic tubes from each control and diabetic patients. About 2 ml blood was placed into ethylene diamine tetra acetic acid (EDTA) vacutainer tube to perform HbA1c and complete blood count (CBC) for cases and controls. The remainder quantity of blood (4 ml) was placed in plastic 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 glucose, insulin, cholesterol, triglycerides, LDL-C, HDL-C, AST, ALT, urea and creatinine. *Helicobacter pylori* IgG was determined in serum by ELISA kit.

## 3.7 Biochemical analysis

### 3.7.1 Determination of *Helicobacter pylori*

Serum *H. pylori* IgG was determined by competitive ELISA for the quantitative determination of *H. pylori* IgG in human serum Catalog number 1425-300 IgG Size: 96 wells, Monobind, USA **(Warren and Marshall, 1983)**.

**Principle**

A Sequential ELISA Method (type 1):

The reagents required for the sequential ELISA assay include immobilized antigen, circulating autoantibody and enzyme-linked species-specific antibody. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenous added biotinylated *H. pylori* antigen.

Upon mixing biotinylated antigen, and a serum containing the antibody, reaction results between the antigen and the antibody to form an immune-complex. The interaction is illustrated by the following equation:

$$\text{h-Ab}_{(\text{X-}H.\ pylori)} + {}^{\text{Btn}}\text{Ag}_{(H.\ pylori)} \underset{-a}{\overset{K_a}{\longleftrightarrow}} \text{h-Ab}_{(\text{X-}H.\ pylori)} - {}^{\text{Btn}}\text{Ag}_{(H.\ pylori)}$$

$^{Btn}Ag_{(H.\ pylori)}$ = Biotinylated Antigen (Constant Quantity)

h-$Ab_{(X\text{-}H.\ pylori)}$= Human Auto-Antibody (Variable Quantity)

$Ab_{(X\text{-}H.\ pylori)}$-$^{Btn}Ag_{(H.\ pylori)}$ = Immune Complex (Variable Quantity)

$k_a$ = Rate Constant of Association

$k_{-a}$ = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antigen. This interaction is illustrated below:

h-$Ab_{(X\text{-}H.\ pylori)}$-$^{Btn}Ag_{(H.\ pylori)}$+$Streptavidin_{CW}$ ⟷ immobilized complex (IC)

$Streptavidin_{CW}$ = Streptavidin immobilized on well

Immobilized complex = sandwich complex bound to the solid surface

After the incubation time, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked species-specific antibody (anti-h-IgG, M or A) is then added to the microwells. This conjugates binds to the immune complex that formed.

$IC_{(h\text{-}IgG,M\ or\ A)}$ + $^{ENZ}Ab_{(X\text{-}h\text{-}IgG,\ M\ or\ A)}$ ⇒ $^{ENZ}Ab_{(X\text{-}h\text{-}IgG,\ M\ or\ A)}$ - $IC_{(h\text{-}IgG,\ M\ or\ A)}$

$IC_{(h\text{-}IgG,\ M\ or\ A)}$ = Immobilized Immune complex (Variable Quantity)

$^{ENZ}Ab_{(X\text{-}h\text{-}IgG,\ M\ or\ A)}$ = Enzyme-antibody Conjugate (Constant Quantity)

$^{ENZ}Ab_{(X\text{-}h\text{-}IgG,\ M\ or\ A)}$ - $I.C._{(h\text{-}IgG,\ M\ or\ A)}$ = Ag-Ab Complex (Variable)

The anti-h-IgG, IgM or IgA enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing several different serum references of known antibody activity, a reference curve can be generated from which the antibody activity of an unknown can be ascertained.

**Reagent**

**A. Anti-*Helicobacter pylori* Calibrators – 1ml/vial**

Five (5) vials of references for anti-*H. pylori* at levels of 0**(A)**, 10**(B)**, 25**(C)**, 50**(D)**, and 100**(E)** U/ml of the IgG, IgM or IgA type. Store at 2-8℃. A preservative has been added.

**B. *Helicobacter pylori* Biotin Reagent – 13ml/vial**

One (1) vial of biotinylated inactivated *H. pylori* (IgG, IgM or IgA) in a buffering matrix. A preservative has been added. Store at 2-8℃.

**C. *Helicobacter pylori* Enzyme Reagent – 13ml/vial**

One (1) vial of anti-human IgG, IgM or IgA-horseradish peroxides (HRP) conjugate in a buffering matrix. A preservative has been added. Store at 2-8℃.

**D. Streptavidin Coated Plate – 96 wells**

One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8℃.

**E. Serum Diluent – 20ml**

One (1) vial of serum diluent containing buffer salts and a dye. Store at 2-8℃.

**F. Wash Solution Concentrate – 20ml**

One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8℃.

**G. Substrate A – 7ml/vial**

One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8℃.

**H. Substrate B – 7ml/vial**

One (1) bottle containing hydrogen peroxide (H2O2) in buffer. Store at 2-8℃.

**I. Stop Solution – 8ml/vial**

One (1) bottle containing a strong acid (1N HCl). Store at 2-8℃.

**Specimen collection and preparation**

The specimens shall be blood; serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin.. Allow the blood to clot for

serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8C$^{o}$ for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20C$^{o}$ for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing.

**Test procedure**

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27℃).

1. Format the microplates wells for each serum reference, control and patient specimen to be assayed in duplicate.
2. Pipette 25µl of the appropriate serum reference, control or diluted patient specimen into the assigned well for IgG determination.
3. Add100µl of *H. pylori* Biotin Reagent Solution.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature**.**
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 350µl of wash buffer, decant (blot) or aspirate. Repeat two additional times for a total of 3 washes.
8. Add100µl of *H. pylori* Enzyme Reagent to all wells.
9. Cover and incubate for 30 minutes at room temperature.
10. Repeat steps (6 & 7) as explained above. Add100µl of Working Substrate Solution to all wells.
12. Incubate at room temperature for 15 minutes.
13. Add 50µl of stop solution to each well and swirl the microplate gently for 15-20 seconds to mix.
14. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader.

**Calculation of results**

A reference curve is used to ascertain the concentration of anti-*H. pylori* in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate.
2. Plot the absorbance for each duplicate serum reference versus the corresponding anti-*H. pylori* activity in U/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. Presence of *H. pylori* IgG Confirmed by IgG ≥20U/ml.

## 3.7.2 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 et al., 1971)**.

**Principle**

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.

**Reagents**

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

**Procedure**

**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℃) 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 1-2 cm 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:

$$(R) = Agly / Atot$$

$$\text{Hemoglobin (\%)} = \frac{(R)\ \text{Unknown} \times \text{Hemoglobin Standard (\%)}}{(R)\ \text{Standard}}$$

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.

## 3.7.3 Determination of serum glucose

Serum glucose is determined by glucose-oxidase procedure **(Trinder, 1969)** using Dialab reagent kits.

**Principle**

Determination of glucose after enzymatic oxidation by glucose oxidase. The colorimetric indicator is quinoneimine, which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidase.

$$\text{Glucose} + O_2 \xrightarrow{GO} \text{Gluconic acid} + H_2O_2$$

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

**Reagents**

| **Reagent** | **Concentration** |
|---|---|
| Phosphate buffer (pH 7.5)<br>Phenol<br>4-Aminoantipyrine<br>Glucose oxidase (GOD)<br>Peroxidase (POD) | 250 mmol/l<br>5 mmol/l<br>0.5 mmol/l<br>≥ 15 ku/l<br>≥ 1 ku/l |
| **Standard** | 100 mg/dl |

**Assay procedure**

Wavelength: 500 nm

Optical path: 1 cm

Temperature: 37 °C

Measurement: Against reagent blank.

- 10 µl of standard (sample or control) was added to 1ml of the reagent and mixed well.
- The mixture was incubated for 10 min at 37 ºC.
- The absorbance was measured within 60 min.

**Calculation**

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

**Reference value** (fasting glucose)

**(Palestinian clinical laboratory tests guide, PCLTG, 2005)**

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

## 3.7.4 Determination of serum insulin

Serum insulin is determined by microparticle enzyme immunoassay (MEIA), using Abbott IMx Insulin assay, following the instruction manual **(Travis, 1980 and National Committee for Clinical Laboratory Standards, 2001)**.

**Biological principles of the procedure:**

The IMx insulin assay was used. It is based on the MEIA technology. The IMx insulin reagents and sample are added to the reaction cell in the following sequence:

1. The probe/electrode assembly delivers the sample, anti-insulin (mouse, monoclonal) coated microparticles and the assay buffer to the incubation well of the reaction cell forming an antibody-insulin complex.
2. An aliquot of the reaction mixture containing insulin bound to the anti-insulin coated microparticles is transferred to the glass fiber matrix.
3. The matrix is washed to remove unbound materials.
4. The anti-insulin: alkaline phosphatase conjugate is dispensed onto the matrix and binds to the antibody-antigen complex.
5. The matrix is washed to remove unbounded materials.
6. The substrate, 4-methylumbelliferyl phosphate, is added to the matrix and the fluorescent product is measured by the microparticle enzyme immunoassay optical assembly.

**Reagents**

Reagent pack

IMx Insulin Reagent Pack, 100 tests (2A10-20)

- 1 bottle (7ml) anti-insulin (mouse, monoclonal) coated microparticles in buffer with protein stabilizers. Preservative: contain sodium azide and antimicrobial agents.
- 1bottle (9ml) Anti-Insulin (Mouse, Monoclonal): alkaline phosphatase conjugate in buffer with protein stabilizers. Minimum concentration: 3µg/ml.
- 1 bottle (10ml) 4-methylumbelliferyl phosphate, 1.2mM, in buffer.
- 1 bottle (14ml) assay buffer in calf serum.

Preservative: All of the above mentioned reagents are contain sodium azide and antimicrobial agents.

**Calculation**

To convert control ranges to the alternate units, perform the following calculations: Concentration in µIU/ml = Concentration in µU/ml x 1.0

Concentration in pmol/L = Concentration in µU/ml x 7.175

## 3.7.5 Determination of serum cholesterol

Enzymatic colorimetric method for the quantitative determination of total cholesterol in serum or plasma, using Diasys Diagnostic Systems, Germany **(Meiattini et al., 1978)**.

**Principle**

Determination of cholesterol after enzymatic hydrolysis and oxidation. The colorimetric indicator is quinoneimine which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidase.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{CHE}} \text{cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHO}} \text{cholesterol-3-one} + H_2O_2$$

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

**Reagents**

Concentrations are those in the final test mixture.

| **Reagent** | **Concentration** |
|---|---|
| Good's buffer (pH 6.7) | 50 mmol/l |
| Phenol | 5 mmol/l |
| 4- Aminoantipyrine | 0.3 mmol/l |
| Cholesterol esterase (CHE) | ≥ 200 u/l |
| Cholesterol oxidase (CHO) | ≥ 100 u/l |
| Peroxidase (POD) | ≥ 3 ku/l |
| **Standard** | 200 mg/dl |

**Assay procedure**

Wavelength: 500 nm

Optical path: 1cm

Temperature: 37 ºC

Measurement: against reagent blank.

- Ten µl of standard (sample or control) was added to 1ml of working reagent and mixed well.
- The mixture was incubated for 5 min at 37 ºC.
- The absorbance was measured within 60 min.

**Calculation**

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

**Reference value**

| | |
|---|---|
| Child (desirable) | < 170 mg/dl |
| Adult (desirable) | <200 mg/dl |

## 3.7.6 Determination of serum triglycerides

Enzymatic colorimetric method for the quantitative determination of triglycerides in serum or plasma, using Diasys Diagnostic Systems, Germany **(Bucolo and David, 1973)**.

**Principle**

Determination of triglycerides after enzymatic splitting with lipoprotein lipase. Indicator is quinoneimine which is generated from 4-aminoantipyrine and 4-chlorophenol by hydrogen peroxide under the catalytic action of peroxidase.

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

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

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

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

**Reagents**

Concentrations are those in the final test mixture.

| **Reagent** | **Concentration** |
|---|---|
| Cood's buffer (pH 7.2) | 50 mmol/l |
| 4-Chlorophenol | 4 mmol/l |
| ATP | 2 mmol/l |
| $Mg^{2+}$ | 15 mmol/l |
| Glycerokinase (GK) | ≥ 0.4 KU/l |
| Peroxidase (POD) | ≥ 2 KU/l |
| Lipoprotein lipase (LPL) | ≥ 2 KU/l |
| 4-Aminoantipyrine | 0.5 mmol/l |
| Glycerol-3-phosphate-oxidase (GPO) | ≥ 0.5 KU/l |
| **Standard** | 200 mg/dl |

**Assay Procedure**

Wavelength: 500 nm

Optical path: 1 cm

Temperature: 37 °C

Measurement: Against reagent blank.

- Ten µl of standard (sample or control) was added to 1ml of working reagent and mixed well.
- The mixture was incubated for 5 min at 37 ºC.
- The absorbance was measured within 60 min.

**Calculation**

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

**Reference value**

| | |
|---|---|
| Child (desirable) | 30 - 150 mg/dl |
| Adult (desirable) M<br>F | 40 - 160 mg/dl<br>35 - 135 mg/dl |

## 3.7.7 Determination of serum high density lipoprotein cholesterol

Liquid HDL-C precipitant 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.

**Reagents**

| **Reagent** | **Concentration** |
|---|---|
| Monoreagent contain: Magnesium chloride<br>Phosphotungstic acid | 1.4 mmol/l<br>8.6 mmol/l |
| **Choesterol standard** | 200 mg/dl |

**Assay procedure**

**1- Precipitation**

- Two hundred µl of standard (sample or control) were added to 500 µl of the precipitation reagent and mixed well.
- The mixture was allowed to stand for 15 min at room temperature, and then centrifuged for 20 min at 4000 rpm.

**2- Cholesterol determination**

Wavelength: 500 nm

Optical path: 1cm

Temperature: 37 ºC

Measurement: against reagent blank.

- One hundred 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 5min at 37 ºC.
- The absorbance was measured within 45 min.

**Calculation**

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

**Reference value**

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

## 3.7.8 Determination of serum low density lipoproteins cholesterol

LDL-C can be calculated using the empirical relationship of Friedewald **(Grove, 1979)**.

**Principle**

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

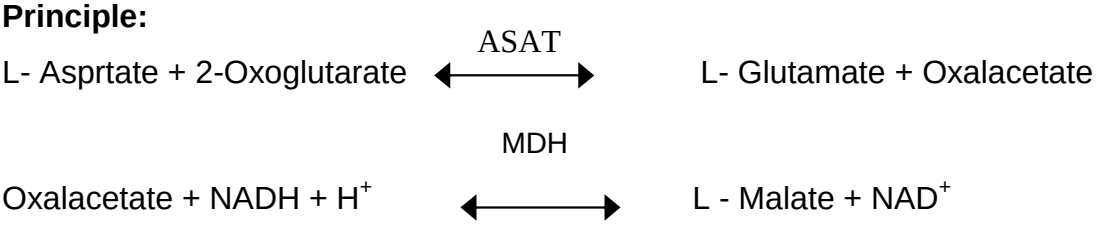
**The Equation**

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

## 3.7.9 Determination of aspartate aminotransferase

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

**Principle:**




**Reagents**

| Reagent | Components | Concentrations |
|---|---|---|
| **Reagent 1** | TRIS pH 7.65<br>L- Aspartate<br>MDH (Malate dehydrogenase)<br>LDH (lactate dehydrogenase) | 80 mmol/l<br>240 mmol/l<br>≥ 600 U/l<br>≥ 900 U/l |
| **Reagent 2** | 2-Oxoglutarate<br>NADH | 12 mmol/l<br>0.18 mmol/l |

**Substrate start**

The reagents are ready to use.

**Sample start**

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 o C & 5 days at 15-25 o C. The monoreagent must be protected from light. The reagent mixture is only prepared just prior to use.

**Procedure**

**Substrate start**

| | |
|---|---|
| Sample | 100µl |
| Reagent 1 | 1000µl |
| Mix, incubate for 5 min., then add: | |
| Reagent 2 | 250µl |
| Mix, read absorbance after 1 min. and start stopwatch. Read absorbance again 1, 2 and 3 min thereafter, at wavelength 340 nm. | |

**Sample start**

| | |
|---|---|
| Sample | 100µl |
| Monoreagent | 1000µl |
| Mix, read absorbance after 1 min. and start stopwatch. Read absorbance again 1, 2 and 3 min thereafter, at wavelength 340 nm. | |

**Calculation**

From absorbance readings calculate ΔA/min and multiply by the corresponding factor from table below:

**ΔA/min x factor = ASAT activity [U/L]**

| | **Substrate start** | **Sample start** |
|---|---|---|
| 340 nm | 2143 | 1745 |
| 334 nm | 2184 | 1780 |
| 365 nm | 3971 | 3235 |

## 3.7.10 Determination of alanine aminotransferase

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

**Principle:**

L- Alanine + 2-Oxoglutarate $\xleftrightarrow[\text{LDH}]{\text{ALAT}}$ L- Glutarate + Pyruvate

Pyruvate + NADH + $H^+$ ⟷ D - Lactate + $NAD^+$

**Reagents**

| Reagent | Components | Concentrations |
|---|---|---|
| **Reagent 1** | TRIS pH 7.5<br>L- Alanine<br>LDH (lactate dehydrogenase) | 100 mmol/l<br>500 mmol/l<br>≥ 1700 U/l |
| **Reagent 2** | 2-Oxoglutarate<br>NADH | 15 mmol/l<br>0.18 mmol/l |

**Substrate start**

The reagents are ready to use.

**Sample start**

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 $^{o}$ C & 5 days at 15-25 $^{o}$ C. The monoreagent must be protected from light. The reagent mixture is only prepared just prior to use.

**Procedure**

**Substrate start**

| | |
|---|---|
| Sample | 100 µl |
| Reagent 1 | 1000µl |
| Mix, incubate for 5 min., then add: | |
| Reagent 2 | 250µl |
| Mix, read absorbance after 1 min. and start stopwatch. Read absorbance again 1, 2 and 3 min thereafter, at wavelength 340 nm. | |

**Sample start**

| | |
|---|---|
| Sample | 100 µl |
| Monoreagent | 1000µl |
| Mix, read absorbance after 1 min. and start stopwatch. Read absorbance again 1, 2 and 3 min thereafter at wavelength 340 nm. | |

**Calculation**

From absorbance readings calculate ΔA/min and multiply by the corresponding factor from table below:

ΔA/min x factor = ALT activity [U/L]

| | **Substrate start** | **Sample start** |
|---|---|---|
| 340 nm | 2143 | 1745 |
| 334 nm | 2184 | 1780 |
| 365 nm | 3971 | 3235 |

## 3.7.11 Determination of serum urea

Serum urea is determined by using colorimetric test **(Fawcett and Scott, 1960)** using DiaSys reagent kits.

**Principle**

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

$$\text{2-Oxoglutarate} + NH_4 + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-Glutamate} + NAD^+ + H_2O$$

**Reagents**

Concentrations are those in the final test mixture.

| **Reagent** | **Concentration** |
|---|---|
| **R1:** TRIS | 120 mmol/l |
| 2- Oxoglutarate | 7 mmol/l |
| ADP | 0.6 mmol/l |
| Urease | ≥ 0.6 ku/l |
| GLDH | ≥ 1 ku/l |
| **R2:** NADH | 0.25 mmol/l |
| **Standard** | 50 mg/dl |

**Assay procedure**

The working solution was prepared by mixing 4 parts of R1 with 1 part of R2.

Wavelength: 340 nm

Optical path: 1cm

Temperature: 37 ºC

Measurement: against distilled water.

- Ten microlitters of standard (sample or control) was added to 1ml of working reagent and mixed well.
- The mixture was incubated for 30 sec then absorbance (A1) was recorded.
- After exactly further 60 sec the absorbance (A2) was measured.

**Calculation**

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

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

**Reference value**

**(PCLTG, 2005)**

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

## 3.7.12 Determination of serum creatinine

Serum creatinine was determined by using kinetic test without deproteinization according to Newman and Price method **(Newman and Price, 1999)** using DiaSys reagent kits.

**Principle**

Creatinine forms a colored orange-red complex in an alkaline picrate solution. The difference in absorbance at fixed times during conversion is proportional to the concentration of creatinine in the sample.

Creatinine + Picric acid $\longrightarrow$ creatinine picrate complex

**Reagents**

Concentrations are those in the final test mixture.

| **Reagent** | **Concentration** |
|---|---|
| **R1:** Sodume hydroxide (pH approx. 13) | 0.16 mol/l |
| **R2:** Picric acid (pH approx. 1.2) | 4.0 mmol/l |
| **Standard** | 2.0 mg/dl |

**Assay procedure**

The working solution was prepared by mixing 4 parts of R1 with 1 part of R2.

Wavelength: 490 nm

Optical path: 1cm

Temperature: 37 ºC

Measurement: against distilled water.

- Fifty microlitters of standard (sample or control) was added to 1ml of working reagent add and mixed well.
- The Mixture was incubated for 60 sec then absorbance(A1) was recorded.
- After exactly further 120 sec the absorbance (A2) was measured.

**Calculation**

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

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

**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<br>F | 0.6 - 1.2 mg/dl<br>0.5 -1.1 mg/dl |

## 3.8 Hematological parameters

A complete system of reagents of control and calibrator, Cell-Dyne 1700 was used to determine the following hematological parameters: white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb) and platelet (PLT) content.

## 3.9 Statistical analysis

Data were computer analyzed using SPSS/ PC (Statistical Package for the Social Science Inc. Chicago, Illinois USA, version 18.0) statistical package.

- Simple distribution of the study variables and the cross tabulation were applied.
- Chi-square ($\chi^2$) 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.
- 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 insulin hormone.
- 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)) * 100.
- Microsoft Excel program version 11.0 was used for graph plotting.

# Chapter 4

# Results

## 4.1 Personal profile of the study population

The present study is a case control design. The study population comprised 90 apparently healthy controls (45 males and 45 females) and 90 type 2 diabetic patients who represent cases (45 males and 45 females). Table 4.1 illustrates personal profile of the study population. Age classification showed that 33 (36.6%) controls and 30 (33.3%) cases were ≤45 years old. Age group 46-55 years comprised 36 (40.0%) controls and 37 (41.1%) cases. Controls and cases aged >55 years old were 21 (23.3%) and 23 (25.5%), respectively. The difference between controls and cases in term of age distribution was not significant ($\chi^2$=0.247 P=0.884). The mean ages of controls and cases were 49.2±6.8 and 49.5±6.6 years old with ranges of 38-61 and 39-62 years, respectively. The independent sample t-test also showed no significant difference between mean ages of controls and cases (t=0.346, P=0.730). Analysis of the educational status of the study population showed that 24 (26.7%) controls and 14 (15.5%) cases had a university degree, 21 (23.3%) and 24 (26.7%) finished secondary school, 15 (16.7%) and 12 (13.3%) passed preparatory school, 12 (13.3%) and 24 (26.7) finished primary school, and 18 (20.0%) and 16 (17.8%) were illiterate. The difference between various educational levels of controls and cases was not significant ($\chi^2$=7.283, P=0.122).

**Table 4.1 Personal profile of the study population.**

| Personal profile | Controls (n=90) | | Cases (n=90) | | Test | | P-value |
|---|---|---|---|---|---|---|---|
| | n | % | n | % | | | |
| **Age (Year)** | | | | | | | |
| ≤45 | 33 | 36.6 | 30 | 33.3 | $\chi^2$ | 0.247 | 0.884 |
| 46-55 | 36 | 40.0 | 37 | 41.1 | | | |
| >55 | 21 | 23.3 | 23 | 25.5 | | | |
| Mean±SD | 49.2±6.8 | | 49.5±6.6 | | **T** | 0.346 | 0.730 |
| Range *(min-max)* | *(38-61)* | | *(39-62)* | | | | |
| **Gender** | | | | | | | |
| Male | 45 | 50.0 | 45 | 50.0 | $\chi^2$ | 0.000 | 1.000 |
| Female | 45 | 50.0 | 45 | 50.0 | | | |
| **Education** | | | | | | | |
| University | 24 | 26.7 | 14 | 15.5 | $\chi^2$ | 7.283 | 0.122 |
| Secondary school | 21 | 23.3 | 24 | 26.7 | | | |
| Preparatory school | 15 | 16.7 | 12 | 13.3 | | | |
| Primary school | 12 | 13.3 | 24 | 26.7 | | | |
| Illiterate | 18 | 20.0 | 16 | 17.8 | | | |

P>0.05: Not significant. n: number of cases and controls.

## 4.2 Socioeconomic data of the study population

Table 4.2 provides socioeconomic data of the study population. The employed controls and cases were 24 (26.7%) and 17 (18.9%) whereas 66 (73.3%) controls and 73 (81.1%) cases were unemployed. The difference between the two groups was not significant ($\chi^2$=1.548, P=0.213). Regarding family income\month, the number of cases with low income was higher than that of controls. The difference between the two groups was significant ($\chi^2$=9.218, P=0.010), implying that family income is associated with diabetes. In addition, family history revealed that, 24 (26.7%) controls and 57 (63.3%) cases reported that they have family history of diabetes, whereas 66 (73.3%) controls and 33 (36.7%) cases did not have family history of diabetes. The difference between the two groups was significant ($\chi^2$=24.444, P=0.000) indicating that, family history is associated with diabetes. Smoking was not found to be associated with diabetes ($\chi^2$=0.887, P=0.346).

**Table 4.2 Socioeconomic data of the study population.**

| Socioeconomic data | Controls (n=90) | | Cases (n=90) | | $\chi^2$ | P-value |
|---|---|---|---|---|---|---|
| | n | % | n | % | | |
| **Employment** | | | | | | |
| Yes | 24 | 26.7 | 17 | 18.9 | 1.548 | 0.213 |
| No | 66 | 73.3 | 73 | 81.1 | | |
| **Family income/month (NIS)*** | | | | | | |
| <1000 | 15 | 16.7 | 32 | 35.6 | 9.218 | 0.010 |
| 1000-2000 | 45 | 50.0 | 30 | 33.3 | | |
| >2000 | 30 | 33.3 | 28 | 31.1 | | |
| **Family history of diabetes** | | | | | | |
| Yes | 24 | 26.7 | 57 | 63.3 | 24.444 | 0.000 |
| No | 66 | 73.3 | 33 | 36.7 | | |
| **Smoking** | | | | | | |
| Yes | 15 | 16.7 | 20 | 22.2 | 0.887 | 0.346 |
| No | 75 | 83.3 | 70 | 77.8 | | |

* NIS: New Israeli Shekels. n: number of cases and controls. P>0.05: Not significant, P<0.05: Significant.

## 4.3 Physical activity, diet and compliance of medication among the study population

Physical activity, diet and compliance of medication among the study population are illustrated in Table 4.3. Although the number of case who doing exercise 43 (18.9%) was lower than controls 48 (26.7%), the difference between the two group was not significant ($\chi^2$=0.556 and P=0.456). Concerning diet, the number of controls and cases who was on diet were 6 (6.7%) and 73 (81.1%), respectively, the difference between the two groups was significant ($\chi^2$=101.268, P=0.000), indicating that, most cases followed diet. In addition, the majority of cases 86 (95.6%) were found to be compliance of medication.

**Table 4.3 Physical activity, diet and compliance of medication of the study population.**

| Item | Controls (n=90) | | Cases (n=90) | | $\chi^2$ | P-value |
|---|---|---|---|---|---|---|
| | n | % | n | % | | |
| **Physical activity** | | | | | | |
| Yes | 48 | 26.7 | 43 | 18.9 | 0.556 | 0.456 |
| No | 42 | 73.3 | 47 | 81.1 | | |
| **Diet** | | | | | | |
| Yes | 6 | 6.7 | 73 | 81.1 | 101.268 | 0.000 |
| No | 84 | 93.3 | 17 | 18.9 | | |
| **Compliance of medication** | | | | | | |
| Yes | - | - | 86 | 95.6 | - | - |
| No | - | - | 4 | 4.4 | - | - |

P>0.05: Not significant, P<0.05: Significant. n: number of cases and controls.

## 4.4 Distribution of diabetic patients by the duration of the disease

Table 4.4 demonstrates the distribution of diabetic patients by the duration of the disease. Patients with diabetes since less than 5 years were 49 (54.4%), whereas those with diabetic duration of 5-10 years were 25 (27.8%).The rest of patients 16 (17.8%) had diabetes for more than 10 years.

**Table 4.4. Distribution of diabetic patients (n=90) by the duration of the disease.**

| Duration of diabetes (Year) | n | % of cases |
|---|---|---|
| **< 5** | 49 | 54.4 |
| **5-10** | 25 | 27.8 |
| **>10** | 16 | 17.8 |

n: number of diabetic patients.

## 4.5 Self-reported complications of the study population

The main self-reported complications among cases and controls are summarized in Table 4.5. The percentages of cardiovascular disease, retinopathy and neuropathy were higher in cases compared to controls (28.9, 63.3 and 34.4 vs 3.3, 4.4 and 2.2%, respectively) with significant differences ($\chi^2_{(corrected)}$=19.895, P=0.000, $\chi^2_{(corrected)}$=67.051, P=0.000 and $\chi^2_{(corrected)}$=29.091, P=0.000, respectively).

**Table 4.5 Self-reported complications among the study population.**

| Self-reported complications | Controls (n=90) | | Cases (n=90) | | $\chi^2$ | P-value* |
|---|---|---|---|---|---|---|
| | n | % | n | % | | |
| **Cardiovascular disease** | | | | | | |
| Yes | 3 | 3.3 | 26 | 28.9 | 19.895 | 0.000 |
| No | 87 | 96.7 | 64 | 71.1 | | |
| **Retinopathy** | | | | | | |
| Yes | 4 | 4.4 | 57 | 63.3 | 67.051 | 0.000 |
| No | 86 | 95.6 | 33 | 36.7 | | |
| **Neuropathy** | | | | | | |
| Yes | 2 | 2.2 | 31 | 34.4 | 29.091 | 0.000 |
| No | 88 | 97.8 | 59 | 65.6 | | |

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

## 4.6 Gastritis and peptic ulcer among the study population

Table 4.6 demonstrates the distribution of gastritis and peptic ulcer among the study population. Gastritis was found in 15 (16.7%) controls compared to 27 (30%) cases. The difference between the two group was significant ($\chi^2$=4.472, P=0.034). In addition, only one (1.1%) control reported peptic ulcer compared to 11 (12.2%) cases ($\chi^2_{(corrected)}$=7.232, P=0.007).

**Table 4.6 Gastritis and peptic ulcer among the study population.**

| Item | Controls (n=90) | | Cases (n=90) | | $\chi^2$ | P-value |
|---|---|---|---|---|---|---|
| | n | % | n | % | | |
| **Gastritis** | | | | | | |
| Yes | 15 | 16.7 | 27 | 30 | 4.472 | 0.034 |
| No | 75 | 83.3 | 63 | 70 | | |
| **Peptic ulcer** | | | | | | |
| Yes | 1 | 1.1 | 11 | 12.2 | 7.232 | 0.007* |
| No | 89 | 89.9 | 79 | 87.8 | | |

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

## 4.7 Body mass index of the study population

Table 4.7 provides the BMI of the study population. The mean weight of controls was 88.3±9.2 Kg compared to 87.5±13.4 Kg of cases. The weight difference was not significant (P=0.660) with % difference=0.9%. There was a significant increase in the mean height of controls compared to cases (1.72±0.07 vs 1.66±0.09 m, % difference=3.6%, t=4.907 and P=0.000). Therefore, BMI was significantly increased in cases compared to controls (31.7±4.8 vs 29.9±4.0, % difference=5.8, t=2.635 and P=0.009).

Table 4.7. Body mass index of the study population.

| Anthropometric measurement | Control (n=90) Mean ±SD | Case (n=90) Mean ±SD | % difference | t | P-value |
|---|---|---|---|---|---|
| **Weight (kg)*** *(min-max)* | 88.3±9.2 *(72-112)* | 87.5±13.4 *(60-130)* | 0.9% | 0.441 | 0.660 |
| **Height (m)**** *(min-max)* | 1.72±0.07 *(1.62-1.87)* | 1.66±0.09 *(1.50-1.85)* | 3.6% | 4.907 | 0.000 |
| **BMI***** *(min-max)* | 29.9±4.0 *(24.1-42.2)* | 31.7±4.8 *(22.6-47.8)* | 5.8% | 2.635 | 0.009 |

*Kg: kilogram, ** m: Meter. ***BMI: Body Mass Index (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 BMI≥30.0 were considered obese **(WHO, 2012)**. All values are expressed as mean±SD. n: number of cases and controls. P>0.05: Not significant, P<0.05:Significant.

## 4.8 Glycated hemoglobin, glucose and insulin level of the study population

As indicated in Table 4.8, the mean levels blood HbA1c and serum glucose and insulin in cases were significantly higher than that in controls (8.2±1.7%, 153.7±53.0 mg/dl and 11.6±9.6 μlU/ml vs 5.2±0.7%, 87.0±12.3 mg/dl and 6.8±5.1 μlU/ml, with % differences= 44.8%, 55.4% and 52.2%, t=15.174, 11.639 and 4.127, respectively and P=0.000).

**Table 4.8. blood HbA1c and glucose and insulin levels among the study population.**

| Parameter | Control (n=90) Mean ±SD | Case (n=90) Mean ±SD | % difference | t | P-value |
|---|---|---|---|---|---|
| **HbA1c (%)*** *(min-max)* | 5.2±0.7 *(3.5-6.5)* | 8.2±1.7 *(5.3-12.0)* | 44.8 | 15.174 | 0.000 |
| **glucose (mg/dl)** *(min-max)* | 87.0±12.3 *(65-116)* | 153.7±53.0 *(71-342)* | 55.4 | 11.639 | 0.000 |
| **Insulin (μlU/ml)** *(min –max)* | 6.8±5.1 *(0.5-18.9)* | 11.6 ± 9.6 *(0.30-50.0)* | 52.2 | 4.127 | 0.000 |

*HbA1c: Glycated hemoglobin. n: number of cases and controls. P<0.05: Significant.

## 4.9 Serum lipid profile of the study population

Table 4.9 illustrates serum lipid profile of the study population including cholesterol, triglycerides, LDL-C and HDL-C. The mean levels of cholesterol and triglycerides were found to be higher in cases (201.4±43.3 and 203.8±97.7 mg/dl, respectively) compared to controls (189.0±37.9 and 153.1±45.7 mg/dl, respectively), with % differences of 6.3% and 28.4%, respectively). This increment was statistically significant for cholesterol and triglycerides (t=2.046, P=0.042 and t=4.454, p=0.000, respectively). However, no statistically significant change was found for LDL-C and HDL-C between cases and controls.

**Table 4.9. Lipid profile of the study population.**

| Lipid profile (mg/dl) | Control (n=90) Mean ±SD | Case (n=90) Mean ±SD | % difference | t | P-value |
|---|---|---|---|---|---|
| **Cholesterol** *(min-max)* | 189.0±37.9 *(120-250)* | 201.4±43.3 *(113-281)* | 6.3 | 2.046 | 0.042 |
| **Triglycerides** *(min-max)* | 153.1±45.7 *(65-215)* | 203.8±97.7 *(57-600)* | 28.4 | 4.454 | 0.000 |
| **LDL-C *** *(min-max)* | 118.7±34.9 *(51-178.6)* | 122.2±42.1 *(49-204.2)* | 2.9 | 0.595 | 0.553 |
| **HDL-C **** *(min-max)* | 39.6±9.1 *(30-61)* | 38.5±7.6 *(28-62)* | 2.8 | 0.916 | 0.361 |

*LDL-C: Low density lipoprotein cholesterol, **HDL-C: High density lipoprotein cholesterol. All values are expressed as mean ±SD. n: number of cases and controls. P>0.05: Not significant, P<0.05: Significant.

## 4.10 Liver and kidney functions of the study population

The activities of serum AST and ALT as a marker of liver function as well as the concentrations of serum urea and creatinine as an indicator of kidney function are pointed out in Table 4.10. There were significant elevations in AST and ALT activities in cases compared to controls (36.3±4.7 and 42.4±5.0 U/L vs 16.9±6.0 and 17.8±8.7 U/L, % difference=72.9 and 81.7, t=24.056 and t=23.243, P=0.000, respectively). Similarly, urea and creatinine concentrations were significantly increased in cases compared to controls (47.0±5.3 and 1.06±0.21 mg/dl, vs 31.5±11.8 and 0.88±0.22 mg/dl, % difference=39.5 and 18.6, t=11.283 and t=5.486, P=0.000, respectively).

**Table 4.10. Liver and kidney functions of the study population.**

| Parameter | Controls (n=90) Mean ±SD | Patients (n=90) Mean ±SD | % Difference | t | P-value |
|---|---|---|---|---|---|
| **AST (U/L)** *(min-max)* | 16.9±6.0 *(10.0-30.0)* | 36.3± 4.7 *(30.0-46.0)* | 72.9 | 24.056 | 0.000 |
| **ALT (U/L)** *(min-max)* | 17.8±8.7 *(10.0-38.0)* | 42.4±5.0 *(30.0-50.0)* | 81.7 | 23.243 | 0.000 |
| **Urea (mg/dl)** *(min-max)* | 31.5±11.8 *(15.0-52.0)* | 47.0± 5.3 *(31.0-55.0)* | 39.5 | 11.283 | 0.000 |
| **Creatinine (mg/dl)** *(min-max)* | 0.88±0.22 *(0.60-1.30)* | 1.06±0.21 *(0.60-1.30)* | 18.6 | 5.486 | 0.000 |

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase. n: number of cases and controls. P<0.05: Significant.

## 4.11 Blood parameters of the study population

Table 4.11 illustrates blood parameters of the study population. White blood cell and platelet counts were significantly increased in cases compared to controls (8.0±1.9 and 262.3±61.3 vs 7.0±1.4 and 224.8±43.4, % differences 13.3 and 15.4, t=3.973, t=4.734, P=0.000, respectively). On the other hand, no significant differences were found in RBC count and hemoglobin content between cases and controls.

**Table 4.11 Blood parameters of the study population.**

| Blood parameter | Controls (n=90) Mean ±SD | Patients (n=90) Mean ±SD | % Difference | t | P-value |
|---|---|---|---|---|---|
| **WBC $\times 10^9$/L** *(min-max)* | 7.0±1.4 *(5.4-11.4)* | 8.0± 1.9 *(3.9-12.9)* | 13.3 | 3.973 | 0.000 |
| **RBCs $\times 10^{12}$/L** *(min-max)* | 4.6±0.47 *(4.0-5.8)* | 4.5± 0.49 *(3.4-6.7)* | 2.2 | 0.966 | 0.335 |
| **Hb (g/dl)** *(min-max)* | 12.7±1.3 *(10.6-15.3)* | 12.6±1.4 *(8.8-16.3)* | 0.79 | 0.388 | 0.698 |
| **PLT $\times 10^9$/L** *(min-max)* | 224.8±43.4 *(147-320)* | 262.3± 61.3 *(152-500)* | 15.4 | 4.734 | 0.000 |

WBC: White blood cell, RBC: Red blood cell, Hb: Hemoglobin, PLT: Platelet. n: number of cases and controls. P>0.05: Not significant, P<0.05: Significant.

## 4.12 Distribution of *Helicobacter pylori* IgG among the study population

Distribution of *H. pylori* IgG among the study population is presented in Table 4.12 and Figure 4.1. Thirty three (36.7%) controls were positive for *H. pylori* IgG compared to 65 (72.2%) cases. The difference between the two groups was significant ($\chi^2$=22.937, P=0.000) with higher distribution of *H. pylori* IgG among cases.

**Table 4.12 Distribution of *Helicobacter pylori* IGg among the study population.**

| ***Helicobacter pylori* IgG** | **Controls (n=90)** | **Cases (n=90)** | **$\chi^2$** | **P-value** |
|---|---|---|---|---|
| | **n (%)** | **n (%)** | | |
| ***Helicobacter pylori* IgG**<br>Positive<br>Negative | 33 (36.7)<br>57 (63.3) | 65 (72.2)<br>25 (27.8) | 22.937 | 0.000 |

n: number of cases and controls, P<0.05: Significant.

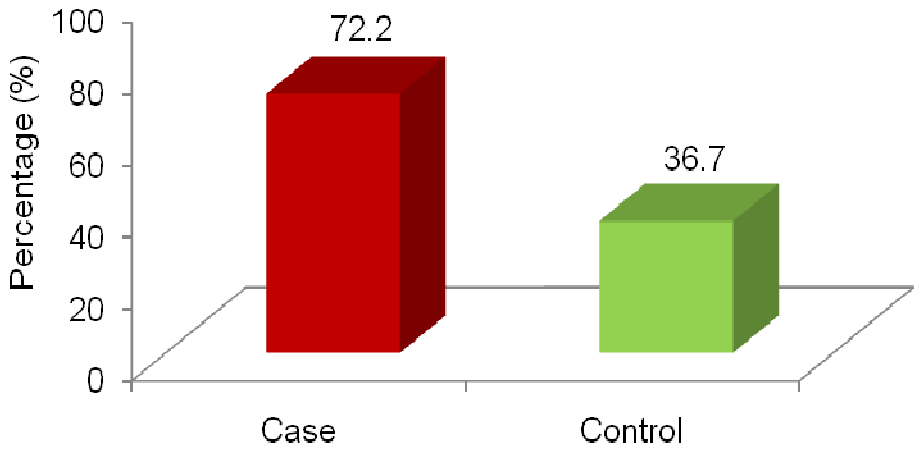



Figure 4.1. Distribution of *Helicobacter pylori* infection among the study population

## 4.13 Relations of *Helicobacter pylori* to different parameters

### 4.13.1 *Helicobacter pylori* in relation to gender among cases

Table 4.13 shows the relationship between gender and *H. pylori* among cases. The prevalence of *H. pylori* among cases was found to be significantly higher in males 37 (82.2%) compared to females 28 (62.2%), $\chi^2$=4.486 and P=0.034, implying that *H. pylori* is associated with gender.

**Table 4.13 *Helicobacter pylori* in relation to gender among cases.**

| ***Helicobacter pylori*** | **Gender** | | **$\chi^2$** | **P-value** |
|---|---|---|---|---|
| | **Male (n=45)** | **Female (n=45)** | | |
| | **n (%)** | **n (%)** | | |
| ***Helicobacter pylori*** | | | | |
| Positive | 37 (82.2) | 28 (62.2) | 4.486 | 0.034 |
| Negative | 8 (17.8) | 17 (37.8) | | |

n: number of males and females. P<0.05:significant.

### 4.13.2 *Helicobacter pylori* in relation to glycated hemoglobin, glucose and insulin of cases.

The relationship between *H. pylori* and blood HbA1c and serum glucose and insulin of cases is illustrated in Table 4.14. The mean level of HbA1c in positive cases was significantly higher than that in negative cases (8.4±1.8 vs 7.6±1.5, P=0.042). However, glucose and insulin levels did not show significant relations with *H. plori* (P>0.05).

**Table 4.14 *Helicobacter pylori* in relation to glycated hemoglobin, glucose and insulin of cases.**

| Parameter | *Helicobacter pylori* | n | Mean ±SD | t | P-value |
|---|---|---|---|---|---|
| **HbA1c (%)** | Positive<br>Negative | 65<br>25 | 8.4±1.8<br>7.6±1.5 | 2.081 | 0.042 |
| **glucose (mg/dl)** | Positive<br>Negative | 65<br>25 | 149.1±47.2<br>165.7±65.3 | 1.342 | 0.183 |
| **Insulin (μlU/ml)** | Positive<br>Negative | 65<br>25 | 12.6 ± 9.6<br>8.9±9.1 | 1.711 | 0.094 |

HbA1c: Glycated hemoglobin. P>0.05: Not significant, P<0.05: Significant.

## 4.13.3 *Helicobacter pylori* in relation to lipid profile of cases

*Helicobacter pylori* in relation to cholesterol, triglycerides, LDL-C, and HDL-C is indicated in Table 4.15. The mean levels of cholesterol, triglycerides, LDL-C in positive cases were significantly higher than that in negative cases (216.4±42.5, 190.1±91.9 and 139.8±42.6 mg/dl, vs 195.6±42.6, 164.5±61.2 and 115.4±40.2 mg/dl, P=0.041, P=0.033 and P=0.013, respectivily). On the other hand the mean level of HDL-C was significantly lower in positive compared to negative cases ( 37.5±6.9 vs 41.1±8.8 mg/dl, P=0.040).

**Table 4.15 *Helicobacter pylori* in relation to lipid profile of cases.**

| Lipid profile (mg/dl) | *Helicobacter pylori* | N | Mean ±SD | t | P-value |
|---|---|---|---|---|---|
| **Cholesterol** | Positive<br>Negative | 65<br>25 | 216.4±42.5<br>195.6±42.6 | 2.078 | 0.041 |
| **Triglyceride** | Positive<br>Negative | 65<br>25 | 190.1±91.9<br>164.5±61.2 | 2.150 | 0.033 |
| **LDL-C*** | Positive<br>Negative | 65<br>25 | 139.8±42.6<br>115.4 ±40.2 | 2.542 | 0.013 |
| **HDL-C**** | Positive<br>Negative | 65<br>25 | 37.5 ±6.9<br>41.1±8.8 | 2.080 | 0.040 |

*LDL-C: Low density lipoprotein cholesterol, **HDL-C: High density lipoprotein cholesterol. n: number of positive and negative cases. P<0.05: Significant.

### 4.13.4 *Helicobacter pylori* in relation to liver and kidney function of cases

Table 4.16 indicates the relationship between *H. pylori* and the liver and kidney functions. The activity of ALT was significantly higher in positive compared to negative cases (43.1±4.9 vs 40.8±4.8 U/L, P=0.049). However AST showed no significant difference between positive and negative cases (36.1±5.0 vs 35.2±2.3 U/L, P=0.252). In addition urea concentration was significantly higher in positive than negative cases (41.1±10.9 vs 37.0±12.8 mg/dl, P=0.022). Although creatinine concentration was higher in positive cases than negative cases, there was no significant difference between the two group (1.0±0.21 vs 0.94±0.25 mg/dl, P=0.058).

**Table 4.16 *Helicobacter pylori* in relation to liver and kidney function of cases.**

| Parameter | *Helicobacter pylori* | n | Mean ±SD | t | P-value |
|---|---|---|---|---|---|
| **AST (U/L)** | Positive<br>Negative | 65<br>25 | 36.1 ±5.0<br>35.2±2.3 | 1.154 | 0.252 |
| **ALT (U/L)** | Positive<br>Negative | 65<br>25 | 43.1±4.9<br>40.8±4.8 | 2.014 | 0.049 |
| **Urea (mg/dl)** | Positive<br>Negative | 65<br>25 | 41.1±10.9<br>37.0±12.8 | 2.315 | 0.022 |
| **Creatinine (mg/dl)** | Positive<br>Negative | 65<br>25 | 1.0±0.21<br>0.94±0.25 | 1.909 | 0.058 |

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase. n: number of positive and negative cases. P>0.05: Not significant, P<0.05: Significant.

### 4.13.5 *Helicobacter pylori* in relation to blood parameters of cases

The relationship between *H. pylori* and blood parameters including WBC, RBC, Hb and PLT is presented in Table 4.17. The WBC count was significantly higher in positive compared to negative cases (8.1±1.8 vs 7.2±1.5 $\times 10^9$/L, P=0.038). However, there were no significant differences in RBC, Hb and PLT between positive and to negative cases (P>0.05).

**Table 4.17 *Helicobacter pylori* in relation to blood parameters of cases.**

| Blood parameter | *Helicobacter pylori* | n | Mean ±SD | t | P-value |
|---|---|---|---|---|---|
| **WBCs $\times 10^9$/L** | Positive | 65 | 8.1±1.8 | 2.146 | 0.038 |
| | Negative | 25 | 7.2±1.5 | | |
| **RBCs $\times 10^{12}$/L** | Positive | 65 | 4.5±0.44 | 0.432 | 0.667 |
| | Negative | 25 | 4.6±0.61 | | |
| **Hb (g/dl)** | Positive | 65 | 12.6±1.5 | 0.481 | 0.633 |
| | Negative | 25 | 12.5±1.1 | | |
| **PLT $\times 10^{12}$/L** | Positive | 65 | 282.9±73.4 | 1.763 | 0.087 |
| | Negative | 25 | 254.4±54.6 | | |

WBC: White blood cell, RBC: Red blood cell, Hb: Hemoglobin, PLT: Platelet. n: number of positive and negative cases. P>0.05: Not significant, P<0.05: Significant.

## 4.13.*6 Helicobacter pylori* in relation to body mass index of cases.

As indicated in Table 4.18 there was no significant difference in BMI of positive compared to negative cases (P=0.160).

**Table 4.18 *Helicobacter pylori* in relation to body mass index of cases.**

| Parameter | *Helicobacter pylori* | n | Mean ±SD | t | P-value |
|---|---|---|---|---|---|
| **BMI** | Positive | 65 | 32.1±5.0 | 1.426 | 0.160 |
| | Negative | 25 | 30.6±4.2 | | |

BMI: Body Mass Index. n: number of positive and negative cases. P>0.05: Not Significant.

# Chapter 5

# Discussion

Diabetes millitus is a multifactorial disease characterized by several metabolic disorders. Its global prevalence rate is alarming. The total number of people with diabetes is projected to rise from 285 million in 2010 to 366 million in 2030 **(Shaw et al., 2010)**. Despite its high prevalence and the subsequent health problems, there are under-diagnosis and under-reporting of diabetes mellitus in the Gaza Strip. Biochemical tests of the disease were restricted to monitoring blood glucose level when the patient visits the clinic. Recently, only one study have been focused on *H. pylori* infection and malnutrition among type 2 diabetic medical services patients in Gaza Strip **(Abu Jabal, 2012)**. The present study is the first to assess *H. pylori* infection in type 2 diabetic patients and its relation to biochemical and hematological parameters. Understanding the role of *H. pylori* in diabetes mellitus could be useful in terms of prognosis and management of the disease.

## 5.1 Sociodemographic data of the study population

The Chi square test showed that, the number of cases with low income was significantly higher than that of controls, implying that, family income is associated with diabetes. This result is in agreement with that reported by **Pilkington et al. (2010)** and **Agardh et al. (2011)**. It is conceivable that the chronic stress of poverty predisposes people to developing diabetes, in light of growing evidence that, poverty is a more important determinant of diabetes and other chronic diseases than lifestyle and other risk factors **(Raphael et al., 2003; Bloch et al., 2008 and Auger and Alix, 2009)**. In this context, the type of purchased food may contribute to the incidence of diabetes **(Liese et al., 2009 and Muraki et al., 2013)**. Regarding family history, the present data revealed that, the number of cases who had family history of diabetes was significantly higher than that of controls. This indicates that, family history is associated with type 2 diabetes. Such finding coincides with that obtained by **Annis et al. (2005); Pijl et al. (2009); Abu Mustafa (2011) and Wagner et al.**

**(2013)**, who reported that, family history is a risk factor for type 2 diabetes. Concerning diet, higher number of cases were found to follow diet than controls, implying that the diabetic patient are aware of the role of diet in the management of the disease. In this context, **Rossi et al. (2013)** reported that a low glycaemic load diet that adequately adheres to the principles of the traditional mediterranean diet may reduce the incidence of type 2 diabetes. **Watkins (2003)** reported that healthy eating is the cornerstone of diabetic treatment, and control of the diet should always be the first treatment offered to Type 2 diabetic patients before drugs are considered.

## 5.2 Diabetes duration and Self-reported complications

The finding that more than half of patients had diabetics since less than 5 years do confirm the idea that type 2 diabetes has long asymptomatic pre-clinical phase which frequently goes undetected. At the time of diagnosis, the patient could have one or more diabetes complications 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 markedly elevated and diabetic complications are developing **(Watkins, 2003)**. The most self-reported symptoms among diabetic patients were retinopathy, neuropathy and cardiovascular disease. Several studies reported similar diabetic complications with increasing rates upon disease progress **(Marshall and Flyvbjerg, 2006 and The National Eye Institute, 2006, Altawil, 2009 and Abu Snayma, 2012)**.

## 5.3 Gastritis and peptic ulcer among the study population

The prevalence of gastritis and peptic ulcer was significantly higher in cases than controls. **Block et al. (2008)** found high prevalence of autoimmune gastritis in diabetic patients. In this context, **Boehme et al. (2007)** concluded that severe acute gastric inflammation or ulcer disease can occur with high prevalence in patients with type 2 diabetes mellitus with little or no dyspeptic symptoms. In addition, **Peng et al. (2013)** documented that type 2 diabetics

had a significantly higher rate of peptic ulcer bleeding than did non diabetic controls.

## 5.4 Distribution of *Helicobacter pylori* among the study population

The results of this study showed significantly higher positive *H. pylori* infection 65 (72.2%) among diabetic patients compared to controls 33 (36.7%). This two-fold increment in *H. pylori* infection in diabetic patients indicates that, *H. pylori* is associated with type 2 diabetes. Higher prevalence of *H. pylori* was found among diabetics patients compared to non diabetics **(Bener, 2007; Devrajani et al., 2010; Abu Jabal, 2012 and Taher et al., 2012)**. When related to gender in type 2 diabetic patients *H. pylori* infection was significantly higher in males than females. Similar results were obtained by **(Kanbay et al., 2005 and Valliani et al., 2013)**. Accordingly, it seems that the male gender is more susceptible to infection and colonization by CagA-positive strains of *H. pylori*. It has been reported that the males are at a greater risk of *H. pylori* clinical manifestations. These observations may account for the higher prevalence of duodenal ulcer and gastric cancer in males **(Jafarzadeh et al., 2007)**.

## 5.5 Body mass index of the study population

In the present study, BMI of cases was significantly higher than that of controls. In other words, obese individuals are at higher risk for diabetes **(Boffetta et al., 2011)**. The literature supported the present results in that obesity is a major risk factor for chronic diseases including diabetes **(Marshall, 2006; Slynkova et al., 2006; Yassin et al., 2011 and Garg et al., 2013)**. It was reported that about 55% of type 2 diabetics are obese **(Eberhart et al., 2004)**. Chronic obesity leads to increased insulin resistance that can develop into diabetes, most likely because adipose tissue is a source of several chemical signals to other tissues **(Akakabe et al., 2013 and Das et al., 2013)**. This was supported by the observed result that insulin level was significantly increased in diabetic patients compared to controls. Other research showed that type 2 diabetes causes obesity as an effect of the

changes in metabolism and other deranged cell behavior attendant on insulin resistance **(Camastra et al., 1999; Bavenholm et al., 2003 and Micic and Cvijocic, 2008)**. In addition, metabolic changes in obesity is characterized by elevation of triglycerides and cholesterol **(Hwang et al., 2006 and Benetti et al., 2013)** which was observed in the present study. When related to *H. pylori* infection, BMI showed no significant association between positive and negative cases.

## 5.6 Glycated hemoglobin, glucose and insulin level of the study population

As indicated in the present results, the mean HbA1c, glucose and insulin levels in cases were significantly higher than that in controls. Similar results were obtained **(Qi et al., 2007; Reznik and Cohen, 2013 and Nicholas et al., 2013)**. In diabetes, prolonged hyperglycemia superdrives nonenzymatic protein glycation, which forms reversible Schiff bases and Amadori compounds. A series of further complex molecular rearrangements then yield irreversible advanced glycosylated end-products (AGEs). AGEs accumulate in the circulating blood and in various tissues **(Furth, 1997 and Yassin et al., 2011)**. It is reported that the levels of HbA1c in the blood reflect the glucose levels to which the erythrocyte has been exposed during its lifespan **(Goldstein, 2004)**. Therefore, the HbA1c test is attractive as it measures chronic glycaemia, rather than instantaneous blood glucose levels. HbA1c has been used as an objective marker of average glycaemic control for many years, has an accepted place in the monitoring of patients with diabetes, and is relied on for significant management decisions, such as initiation of insulin therapy **(d´Emden et al., 2012)**. Hyperinsulinemia recorded in the present study indicated the development of insulin resistance in diabetic patient, which minimize the utilization of glucose by the cell leading to hyperglycemia. Insulin resistance was a well established feature in type 2 diabetes **(ADA, 2013 and Meier and Bonadonna, 2013)**. In diabetic patient *H. pylori* infection was found to be associated with HbA1c. This result is in agreement with that obtained by **Chen and Blaser (2012) and Hsieh et al. (2013)**. One potential biological mechanism that might explain the link between *H. pylori* infection

and HbA1c levels is related to the role of *H. pylori* in the host metabolic homeostasis by affecting the production of gastric hormones such as ghrelin and leptin **(Osawa, 2008; Pacifico et al., 2008 and Francois et al., 2011)**. These hormones are involved in the regulation of appetite and energy expenditure. The other suggested mechanism is that cytotoxin-associated gene A (cagA) protein produced by *H. pylori* could be an important contributor to the inflammatory disorders involved in the metabolic syndrome **(Atherton, 2006 and Cohen and Muhsen, 2012)**.

## 5.7 Lipid profile of the study population

The present results demonstrate significant increase in cholesterol and triglyceride levels of cases compared to controls. On the other hand, no significant differences were found in LDL-C and HDL-C levels between cases and controls. Elevation of cholesterol and triglycerides in diabetic patients was documented by several authors **(Chen et al., 2009; Katsiki et al., 2011; von Eckardstein and Sibler, 2011 and Al–Hakeim and Ali, 2012)**. 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, since elevation of insulin inhibits the hormone sensitive lipase. 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 *H. pylori*, cholesterol, triglycerides and LDL-C levels were significantly higher in positive than in negative cases, whereas HDL-C level was significantly lower in positive cases. Similar results were previously reported **(Ugwu et al., 2008; El Hadidy et al., 2009 and Tanriverd, 2011)**. The mechanism of how *H. pylori* infection modifies the serum lipid profiles is still not clear, but a plausible explanation is that systemic inflammatory response to the bacterium induces changes in lipid and lipoprotein metabolism **(Khovidhunkit et al., 2000)**. That is, chronic *H. pylori* infection has been postulated to shift the lipid profile toward an atherogenic direction via the action of proinflammatory cytokines, such as interleukins 1 and 6, interferon-alpha, and tumor necrosis factor alpha (TNF-α). These cytokines are capable of affecting lipid metabolism in various ways, including activation of adipose tissue lipoprotein lipase, stimulation of

hepatic fatty acid synthesis, influencing lipolysis and the increasing hepatic Hydroxyl methy glutary-CoA (HMG-CoA) reductase activity **(Khovidhunkit et al., 2004)**. Thus, *H. pylori* infection could play a role in the atherosclerotic process and may be a reliable indicator for the assessment of cardiovascular disease risk **(Lim et al., 2013)**. This is supported the self-reported complication of cardiovascular disease reported in the present study.

## 5.8 Liver and kidney functions of the study population

In the present study, liver function was assessed through determination of AST and ALT. There were marked elevations in AST and ALT activities. Such finding are in agreement with the previous studies **(Forlani et al., 2008 and Saligram et al., 2012)**. Aspartate aminotransferase and ALT were significantly elevated in cases compared with controls, 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 **(Cho et al., 2007 and Atiba et al., 2013)**. Other potential explanations for elevated transaminases in insulin-resistant states include oxidantive stress from reactive lipid peroxidation, peroxisomal beta-oxidation, and recruited inflammatory cells. The insulin resistant state is also characterized by an increase in pro-inflammatory cytokines such as TNF-α, which may also contribute to hepato-cellular injury **(Good et al., 2006)**. It is also hypothesized that the elevated ALT, a gluconeogenic enzyme whose gene transcription is suppressed by insulin, could indicate an impairment in insulin signalling rather than purely hepatocyte injury **(Villegas et al., 2011 and Atiba et al., 2013)**. However, ALT elevation could also have been due to overweight **(Harris, 2005)**, but in our study both cases and controls are overweight.

Serum urea and creatinine concentrations of diabetic patient were significantly increased compared to that of controls. Urea is formed by the liver as an end product of protein breakdown and is one marker of the kidney function **(Rajagopalan et al., 2013)**. 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)**. When related to *H. pylori* infection, ALT and urea were found to be significantly higher in positive compared to negative cases. Multivariate logistic regression analysis and a population study of end stage renal disease patients revealed that serum urea was significantly associated with *H. pylori* infection **(Khedmat et al., 2007 and Jalalzadeh et al., 2011)**. In addition, **Takuma (2011)** demonstrated that *H. pylori* infection was one of the independent risk factors for the development of liver disease. In this context, it is known that the level of ALT activity reflects damage to hepatocytes and is considered to be a highly sensitive and fairly specific preclinical and clinical biomarker of hepatotoxicity **(Ozer et al., 2008)**.

## 5.9 Hematological profile of the study population

White blood cell and platelet counts were significantly increased in cases compared to controls whereas no significant change was found in RBC count and hemoglobin content between cases and controls. Leuckocytosis and thrombocytosis were reported in diabetic patients **(Charles et al., 2007 and Farhangi et al., 2013)**. In addition, **Chen et al. (2006)** concluded that elevated WBC count but not RBC count was significantly associated with insulin resistance and glycaemic metabolism. The insulin resistant state is characterized by an increase in pro-inflammatory cytokines, which may contribute to leuckocytosis **(Good et al., 2006 and Farhangi et al., 2013)**. Platelets hyperactivity may stand behind the detected thrombocytosis in diabetic patients **(Demirtunc et al., 2009 and Kodiatte et al., 2012)**. When related to *H. pylori*, the WBC count was significantly higher in positive compared to negative cases. leukocytosis was reported in *H. pylori* infected patients **(Tanriverd, 2011 and Jafarzadeh et al., 2013)**. The elevation of WBC in *H. pylori* observed in infected cases may be attributed to increase production of inflammatory cytokines such as interleukin-8, interleukin-6, and TNF-$\alpha$ from epithelial cells in the gastric mucosa **(Iida et al., 2012)**.

# Chapter 6

# Conclusions & Recommendations

## 6.1 Conclusions

1. Diabetes mellitus was more prevalent among families with low income as well as among individuals with family history of the disease.

2. More than half of patients had diabetes since less than 5 years and most of them followed diet.

3. The main self-reported complications among diabetic patients were retinopathy, neuropathy and cardiovascular disease. In addition, the prevalence of gastritis and peptic ulcer was significantly higher among cases compared to controls.

4. The BMI was significantly higher in cases than controls.

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

6. Serum cholesterol and triglycerides were significantly increased in cases compared to controls.

7. The activities of serum AST and ALT, and the concentrations of serum urea and creatinine were significantly elevated in cases in comparison with controls.

8. The WBC and PLT counts were significantly higher in cases than controls.

9. The prevalence of *H. pylori* in diabetic patients was significantly higher than in controls. Infection with *H. pylori* was significantly higher in diabetic males than diabetic females.

10. When related to *H. pylori*, blood HbA1c levels were significantly higher in positive than in negative cases.

11. Serum cholesterol, triglycerides and LDL-C levels were significantly increased in *H. pylori* positive cases more than in negative cases, whereas HDL-C level was significantly lower in positive cases.

12. The activity of serum ALT and the concentration of urea were significantly increased in *H. pylori* positive cases compared to negative cases.

13. The WBC count was singnificantly elevated in *H. pylori* positive cases compared to negative cases.

## 6.2 Recommendations

1. Frequent monitoring of *H. pylori* infection as a risk factor of type 2 diabetes, is recommended.

2. Estimation of lipid profile is needed to avoid the deleterious effect of *H. pylori* infection associated with diabetes.

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

4. Further research is highly recommended on *H. pylori* infection among type 1 diabetes and other chronic diseases.

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# Annex 1: Ministry of Health permission letter

الجـــــــــامعة الإسلامية – غزة كلية العلوم
The Islamic University of Gaza
منسق برنامج ماجستير العلوم الحياتية

التاريخ/ 2013/07/31م

الأخ/ د. ناصر الدين ابوشعبان مدير عام التنمية البشرية بوزارة الصحة حفظه الله ،،،

السلام عليكم ورحمة الله وبركاته ،،،

**الموضوع / تسهيل مهمة باحث**

تشهد إدارة ماجستير العلوم الحياتية بالجامعة الإسلامية أن الطالب: ***نبيل محمد جمال سعدالله*** طالب في برنامج ماجستير العلوم الحياتية تخصص –***أحياء دقيقة*** يقوم بإجراء البحث النهائي في برنامج الماجستير والذي بعنوان:

**Assessment of *Helicobacter pylori* Infection as a risk factor for type 2 diabetes mellitus in Gaza strip**

الباحث بحاجة للحصول علي المعلومات والبيانات وعينات من العيادة الخارجية بمجمع الشفاء الطبي.

لذا نرجو من سيادتكم مساعدة الباحث في الحصول علي المعلومات والبيانات والعينات.

**ولكم منا جزيل الشكر والتقدير ،،،**

-مرفق لكم نسخة من البحث

منسق برنامج ماجستير العلوم الحياتية
د. محمد فؤاد أبوعودة

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

# Annex 2: Helsinki committee an approval letter

الجـــــــــامعة الاســـــلامية – غزة
The Islamic University of Gaza
منسق برنامج ماجستير العلوم الحياتية

كلية العلوم

التاريخ: 2013/07/31م

السادة أعضاء هيئة هلسنكي للبحوث حفظهم الله،،،
وزارة الصحة – غزة – فلسطين
السلام عليكم ورحمه الله وبركاته،،،

**الموضوع / موافقة على بحث**

نرجو من سيادتكم الموافقة على البحث المقدم من قبل الباحث / ***نبيل محمد جمال سـعدالله***،
وذلك ضمن برنامج ماجستير العلوم الحياتية التابع لكلية العلوم – الجامعة الإسلامية.

**وهو بعنوان:**

**Assessment of *Helicobacter pylori* Infection as a risk factor for type 2 diabetes mellitus in Gaza strip**

سيجرى البحث في بداية أغسطس 2013 .

**لكم منا جزيل الشكر والامتنان،،،**

– مرفق لكم نسخة من البحث.

منسق برنامج ماجستير العلوم الحياتية
د.محمد فؤاد أبوعودة

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

## Annex 3: Questionnaire

Case control study, questionnaire for assessment of *Helicobacter pylori* infection as a risk factor for type 2 diabetes mellitus in 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 بالجرثومة الملوية البوابية (*Helicobacter pylori*). 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: ☐Yes ☐No
- Smoking: ☐Yes ☐No
- Physical activity: ☐Yes ☐No
- Diet: ☐Yes ☐No
- Compliance of medication **(only for patients)**: ☐Yes ☐No
- Duration of DM2/years: **….(only for patients)**

## 3. Self-reported complications:

| | | |
|---|---|---|
| Cardiovascular diseases | ☐Yes | ☐No |
| Retinopathy | ☐Yes | ☐No |
| Neuropathy | ☐Yes | ☐No |
| Gastritis | ☐Yes | ☐No |
| Peptic ulcers | ☐Yes | ☐No |

## 4. Anthropometric measurement:

Height (cm): ……...Weight (kg):……

Body Mass Index: ……..

**Agreement:**
I agree to complete this questionnaire concerning my health statement.
أنا موافق على تعبئة هذا الاستبيان الذي يتعلق بصحتي.

التوقيع: .....
التاريخ: .....

شكرا لكم على حسن تعاونكم

**الباحث/ نبيل محمد سعدالله**