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Helicobacter pylori infection among type 1 diabetic children in Gaza strip

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By

Eslam S. ALsharif

Supervisor

Prof. Dr. Maged M. Yassin

Professor of Physiology

Faculty of Medicine

The Islamic University of Gaza

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أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

العدوى ببكتريا الملوية البوابية بين الاطفال المصابين بالسكري النوع الاول في قطاع غزة

Helicobacter pylori infection among type 1 diabetic children in Gaza strip

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نتيجة الحكم على أطروحة ماجستير

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العدوى ببكتيريا الملوية البوابية بين الأطفال المصابين بالسكري من النوع الأول في قطاع غزة

Helicobacter pylori infection among type 1 diabetic children in Gaza strip

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

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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.

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

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Helicobacter pylori infection among type 1 diabetic children in Gaza strip

Abstract

Background: Diabetes is one of the leading causes of death in the world and type 1 diabetes usually strikes children and young adults. Over 17 new cases of type 1 diabetes were identified per 100 000 children worldwide annually. *Helicobacter pylori* (*H. pylori)* infection is believed to be associated with Type 1diabetes.

Objective: To assess *H. pylori infection* among type 1 diabetic patients in Gaza strip.

Materials and methods: This case-control study comprised 60 type 1 diabetic children and 60 healthy controls. Questionnaire interview was applied. Blood samples were collected, processed and analyzed for *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), hematocrit (Hct) and platelet (PLT) were determined. Data were analyzed using SPSS version 18.0.

Results: Type 1 diabetes was more prevalent among families with higher income as well as among individuals with family history of the disease. Three quarters of cases were not on diet. However, the majority of cases were found to be compliance of medication. Almost two-thirds of patients had diabetes since less than 5 years. Neuropathy and gastritis were significantly more frequent among cases compared to controls. The BMI was significantly increased in cases compared to controls (18.6±3.8 versus 17.3±3.3 Kg/m^2, P=0.047). Blood HbA1c and serum glucose and insulin levels were significantly higher in cases compared to controls (9.8±1.8%, 169.6±111.9 mg/dl and 26.7±17.0 mlU/ml versus 5.0±0.5%, 77.7±7.0 mg/dl and 10.6±4.7 mlU/ml, respectively, P=0.000). Serum AST and ALT activities were significantly higher in cases compared to controls (32.1±7.8 and 26.5±12.4 U/L *versus* 22.0±10.5 and 19.5±7.5U/L, respectively, P=0.000). White blood cell count was significantly increased in cases compared to controls (7.5±2.4 versus 6.7±1.8 x 10^9/L, P=0.030). Conversely RBC count and hemoglobin content showed significant decreases in cases than controls (4.5±0.4 and 11.7±1.2 versus 4.7±0.5 x 10^{12}/L and 12.4±1.3 g/dl, P=0.016 and 0.005 respectively). The prevalence of *H. pylori* among diabetic patients 24 (40.0%) was significantly higher than controls 13 (21.7%) with P=0.030. When related to *H. pylori*, BMI showed significant increase in positive cases compered to negative cases (21.0±3.8 *versus*

17.8±3.5, P=0.003). Blood HbA1c and serum glucose and insulin levels were significantly higher in positive than in negative cases (10.5±1.7%, 233.8±145.2 and 34.2±21.7 *versus* 9.5±1.7%, 146.3±87.9 mg/dl and 23.9±14.2 mlU/ml, P=0.046, 0.006 and 0.037 respectively). Serum cholesterol, triglycerides and LDL-C were significantly increased in *H. pylori* positive cases than in negative cases (186.2±35.0, 135.5±44.2 and 107.9±32.9 mg/dl, *versus* 157.8±19.4, 99.5±29.4 and 83.4 ±18.6 mg/dl, P=0.001, P=0.001 and P=0.001, respectivily), whereas HDL-C was significantly lower in positive cases (51.3 ±3.7 *versus* 54.6±2.6 mg/dl, P=0.001). The activity of ALT was significantly higher in positive compared to negative cases (29.1±8.5 versus 24.5±7.3U/L, P=0.045). The WBC count was also singnificantly elvated in *H. pylori* positive cases (7.8±1.5 versus 6.7±1.9 $\times 10^9$/L, P=0.041). Conversely, RBC and Hb were significantly decrease in positive cases than negative cases (4.6±0.5 and 11.9±1.1 versus 4.9±0.4$\times 10^{12}$/L and 13.0±1.4 g/dl, P=0.023 and 0.012 respectivily).

Conclusions: *H. pylori* infection was significantly higher in type 1 diabetic patients compared to controls. *H. pylori* infection was positively associated with BMI, blood HbA1c, serum glucose and insulin, serum cholesterol, triglycerides, LDL-C, ALT, and WBC and nigatively associated with HDL-C, RBC count and Hb content.

Keywords: *Helicobacter pylori*, Type 1 diabetes, Gaza Strip.

العدوى ببكتريا الملوية البوابية بين الاطفال المصابين بالسكري النوع الاول في قطاع غزة

ملخص الدراسة

مقدمة: مرض السكري هو واحد من الأسباب الرئيسية للوفاة في العالم، وداء السكري النوع الأول عادة ما يصيب الأطفال وصغار البالغين. وقد تم تحديد أكثر من 17 حالة جديدة من مرض السكري من النوع 1 لكل 100 000 طفل في جميع أنحاء العالم سنويا. و يعتقد بأن العدوى بالجرثومة الملوية البوابية مرتبطة بسكري النوع الأول.

الهدف: تهدف الدراسة إلى تقييم الإصابة بالجرثومة الملوية البوابية بين الاطفال المصابين بالسكري النوع الأول في قطاع غزة.

الطرق والأدوات: اشتملت هذه الدراسة مجموعة مرضية و مجموعة ضابطة على 60 طفل من مرضى سكري النوع الأول و60 طفل 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 تعداد كل من خلايا الدم البيضاء، خلايا الدم الحمراء، الهيموجلوبين، الهيماتوكريت والصفائح الدموية.

استخدم البرنامج الإحصائي SPSS-18.0 لتحليل البيانات والنتائج.

النتائج: أظهرت النتائج بأن سكري النوع الأول يعتبر أكثر انتشارا بين العائلات الأكثر دخلا بالإضافة إلى الأشخاص ذوي التاريخ العائلي للمرض، كما أظهرت النتائج بأن ثلاثة أرباع الحالات لايتبعون حميه غذائيه ومايقارب من ثلثين الحالات المرضية كانوا حاملين لمرض السكري منذ أن كانت أعمارهم أقل من 5 سنوات. كما أظهرت الدراسة بأن التعقيدات الناتجة عن المرض هي اعتلال اللأعصاب. كما أظهرت النتائج وجود ارتفاع في انتشار التهاب المعدة عند الحالات المرضية مقارنة بالأصحاء، وهذه النتيجة ذات دلالة إحصائية. وأظهرت نتيجه مؤشر كتله الجسم وجود زيادة ذات دلالة إحصائية عند الحالات المرضية مقارنة بالأصحاء (18.6 ± 3.8 مقابل 17.3 ± 3.3 KG/M^2 وقيمة P = 0.047)، وأوضحت النتائج وجود زيادة ذات دلالة إحصائية في مستويات كل من الهيموجلوبين السكري A1C، الجلوكوز والأنسولين عند الحالات المرضية مقارنة بالأصحاء (9.8 ± 1.8% ، 169.6 ± 11.9 MG/DL ، 26.7 ± 17.0 MLU/ML مقابل 5.0 ± 0.5%، 77.7 ± 7.0 MG/DL ، 10.6 ±4.7 MLU/ML وقيمة P = 0.000) . 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(32.1 ± 7.8 و 26.5 ± 2.4 U/L مقابل 22.0 ± 10.5 و19.5± 7.5 U/L وقيمة P = 0.000) كما وجد ارتفاع ذو دلالة إحصائية في أعداد كرات الدم البيضاء عند الحالات المرضية مقارنة مقارنة بالأصحاء (7.5 ± 2.4 مقابل 6.7 ± 1.8 x ^{9}10/L وقيمة P = 0.030) ، وبالعكس أشارت النتائج الى وجود انخفاض في أعداد كرات الدم الحمراء ومحتوى الهيموجلوبين ذات دلالة إحصائية في الحالات المرضية مقارنه بالأصحاء(4.5 ± 0.4 و 1.7± 1.2 مقابل 4.7 ± 0.5 x ^{12}10/L و 12.4 ± 1.3 G/DL وقيمة P = 0.016 و 0.005) وأظهرت النتائج زيادة في انتشار الجرثومة الملوية البوابية بين مرضى السكري24(40.0%) مقارنة بالأصحاء 13(21.7%) وقيمة P = 0.030 وعند عمل علاقة بين المتغيرات مع الجرثومة الملوية البوابية في الحالات المرضيه وجدنا بأن مؤشر كتله الجسم أعلى عند الحالات المرضية المصابين بالجرثومة الملوية البوابية من تلك الحالات المرضية الغير مصابين بالجرثومة الملوية البوابية (21.0 ± 3.8 مقابل 17.8 ± 3.5 و قيمة P = 0.003) ، كذلك مستويات الهيموجلوبين السكري A1C ، الجلوكوز والأنسولين هي أعلى عند الحالات المصابين مقارنه بالغير مصابين (10.5± 1.7% و233.8± 145.2 MG/DL و 34.2 ± 21.7 مقابل 9.5 ± 1.7% و146.3 ± 87.9 MG/DL و 23.9 ± 14.2 MLU/ML وقيمة P = 0.046 و 0.006 و 0.037) ، كما أظهرت النتائج وجود زيادة في مستويات كل من الكلسترول، الدهون الثلاثية، وكلسترول البروتين الشحمي خفيض الكثافة عند الحالات المرضية المصابين أكثر من الحالات الغير مصابين، وهذه النتيجة ذات دلالة إحصائية (186.2± 35.0 و 135.5 ± 44.2 و 107.9 ± 32.9 MG/DL مقابل 157.8 ± 19.4 و99.5 ± 29.4 و 83.4 ± 18.6 MG/DL وقيمة P = 0.001 و0.001و0.001) بينما وجد انخفاض ذو دلالة إحصائية في مستوى كلسترول البروتين الشحمي مرتفع الكثافة عند الحالات المرضية المصابين بالجرثومة. وأشارت النتائج لوجود زيادة في نشاط الإنزيم الناقل للألانين أمين عند الحالات المرضية المصابين بالجرثومة الملوية البوابية مقارنة مع الغير مصابين (51.3±3.7 مقابل54.6±2.6 MG/DL وقيمة P =0.001) ،وهذه الزيادة ذات دلالة إحصائية. كذلك وجد ارتفاع ذو دلالة إحصائية في عدد كرات الدم البيضاء عند الحالات المرضية المصابين بالجرثومة(7.8 ± 1.5مقابل 6.4 ± 1.9 x^{9}10/L وقيمة P =0.041) كذلك انخفاض في أعداد كرات الدم الحمراء ومحتوى الهيموجلوبين ذو دلالة إحصائية عند الحالات المرضية المصابين بالجرثومة مقارنة مع الغير مصابين (4.6 ± 0.5 و 11.9 ± 1.1 مقابل 4.9 ± 0.4 x10^{12}/L و13.0 ± 1.4 G/DL وقيمة P = 0.023 و 0.012) .

الاستنتاجات: أشارت النتائج بأن معدل الإصابة بالجرثومة الملوية البوابية أعلى عند مرضى سكري النمط الأول بالمقارنة مع الأصحاء، وهذه النتيجة ذات دلالة إحصائية، وأظهرت النتائج بأن الإصابة بالجرثومة الملوية البوابية مرتبطه ارتباط طردي مع كل من مؤشر كتله الجسم، الهيموجلوبين السكري A1C، مستويات الجلوكوز والأنسولين، كذلك مستويات الكولسترول، الدهون الثلاثية، وكلسترول البروتين الشحمي خفيض الكثافة، الإنزيم الناقل للألانين أمين وعدد كرات الدم البيضاء. كذلك ترتبط ارتباط عكسي مع كل من كلسترول البروتين الشحمي مرتفع الكثافة، وعدد كرات الدم الحمراء ومحتوى الهيموجلوبين.

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

List of Contents

List of Tables

List of figures

Page

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
CDC	Centers for Disease Control
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
Hct	Hemtocrite
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
LPL	Lipoprotein Lipase
MALT	Mucosa associated lymphoid tissue
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 multifactorial 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)**. The high blood sugar produces the classical symptoms of diabetes mellitus including polyuria, polydipsia and polyphagia **(American Diabetes Association, ADA, 2010).**

Two major types of diabetes were identified; type 1 and type 2. Lack of or severe reduction in insulin secretion due to autoimmune or viral destructions of β cells is responsible for type 1 diabetes, which accounts for 5-10% of diabetic patients **(Harjutsalo et al., 2006)**. Patients with type 1 diabetes usually required insulin injection **(Von Bell et al., 2011)**. Type 2 diabetes accounts for about 90% of cases and usually begins as insulin resistance, a disorder in which the cells do not use insulin properly **(Carrera Boada and Martínez-Moreno, 2013)**.

The autoimmune destruction of pancreatic β-cells, leads to a deficiency of insulin secretion which results in the metabolic derangements associated with type 1 diabetes. In addition to the loss of insulin secretion, the function of pancreatic α-cells is also abnormal and there is excessive secretion of glucagons irrespective of hyperglycemia **(Raju and Raju, 2010).** The resultant inappropriately elevated glucagons exacerbate the metabolic defects. The most pronounced example of this metabolic disruption is that patients with type 1 diabetes rapidly develop diabetic ketoacidosis. Insulin deficiency leads also to uncontrolled lipolysis and elevated levels of free fatty acids in plasma, which suppresses glucose metabolism in peripheral tissues **(Raju and Raju, 2010 and Ozougwu 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 linked to diabetes mellitus. An increased prevalence of *H. pylori* infection among type 1 diabetic patients was documented in developed and developing countries **(EI-Eshmawy et al., 2011; Candelli et al., 2012; Wang et al., 2013 and Fayed et al., 2014)**. In Gaza strip, only three recent studies assessed *H. pylori* infection in type 2 diabetic patients as well as in coronary artery disease patients **(Abu Jabal, 2012; Mansour, 2014 and Yassin et al., 2014).** No previous study linked *H. pylori* to type 1 diabetes. Therefore, the present study is the first to assess *H. pylori* infection among type 1 diabetic children in Gaza strip.

1.2 General objective

To assess *H. pylori* infection among type 1 diabetic children in Gaza strip.

1.3 Specific objectives

1. To determine the prevalence of *H. pylori* infection among cases compared with controls.
2. To assess the level of blood glycated hemoglobin (HbA1c) and, serum glucose and insulin in cases and controls.
3. To measure lipid profile including cholesterol, triglycerides, Low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) in cases versus controls.
4. To estimate liver function through determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as well as kidney function through determination of urea and creatinine.
5. To evaluate blood parameter including white blood cells (WBCs), red blood cells (RBCs), hemoglobin, hematocrite and platelets in cases compared to controls.

6. To verify the relationship between *H. pylori* and the studied parameters in diabetic children.

1.4 Significance

1. Diabetes mellitus is the fifth leading cause of death in Palestine **(Palestinian Ministry of Health, 2011.)**.

2. The present study is the first to assess *H. pylori* infection among type 1 diabetic patients in Gaza strip.

3. 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

World health organization (WHO, 2011) described the term diabetes mellitus as a metabolic disorder with heterogenous etiologies which is characterized by chronic hyperglycaemia and disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The long-term relatively specific effects of diabetes include development of retinopathy, nephropathy and neuropathy. People with diabetes are also at increased risk of cardiac, peripheral arterial and cerebrovascular disease. **American Diabetes Association (2014)** defined diabetes mellitus as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels.

2.2 Types of diabetes

The most common types of diabetes mellitus are:

2.2.1 Type 1 diabetes

This form of diabetes, which accounts for 5-10% of those with diabetes, previously encompassed by the terms insulin dependent 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 **(Ozougwu et al., 2013 and ADA, 2014)**.

2.2.2 Type 2 diabetes

Type 2 diabetes accounts for about 90-95% of all diagnosed cases of diabetes. It is previously referred to as non–insulin dependent diabetes or adult-onset diabetes. The causes of type 2 diabetes are multi-factorial and caused by a group of both genetic and

environmental factors. Type 2 diabetes is characterized by insulin resistance, a disorder in which the cells do not use insulin properly **(Defronzo et al., 2012; Hassan, 2013 and ADA, 2014)**.

2.2.3 Gestational diabetes

Gestational diabetes mellitus refers to glucose intolerance with onset or first recognition during pregnancy **(Goldenberg and Punthakee, 2013).** 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 1 diabetes

2.3.1 Definition and etiology

Type 1 diabetes develops from a cellular-mediated autoimmune destruction of pancreatic B cells resulting in insulin deficiency. The immune system incorrectly manufactures antibodies and inflammatory cells that are directed against and cause damage to patients' own body tissues. It is believed that the predisposition to develop these abnormal antibodies in type 1 diabetes mellitus is, in part, genetically inherited, though the details are not fully understood **(Kantarova and Buc, 2007; Bluestone et al., 2010 and Zhao et al., 2012).** Exposure to certain viral infections or other environmental toxins may serve to trigger abnormal antibody responses that cause damage to the pancreatic cells where insulin is made. These antibodies can be measured in the majority of patients, and may help determine which individuals are at risk for developing type 1 diabetes **(Achenbach et al., 2005 and Aljabri et al., 2013**). In type 1 diabetes, the rate of B-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in other (mainly adults). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stress **(Van Belle et al., 2011 and ADA, 2014).**

2.3.2 Prevalence and mortality rate of type 1 diabetes

Over 17 new cases of type 1 diabetes were identified per 100 000 children worldwide annually. The prevalence rate is particularly high in Finland, Sweden and Norway, with over 25 new cases detected every year per 100 000 children **(Organization for Economic Co-operation and Development, OECD, 2013)**. In Mexico and Korea, the rate is less than five new cases per 100 000 children. While type 1 diabetes currently accounts for only 10-15% of all diabetes cases, there is evidence that incidence rates are rising strongly in some countries. Between 2005 and 2020, new cases of type 1 diabetes for children are expected to double in Europe **(Patterson et al., 2009)**. In 2011 there were almost 660 000 diabetes related death worldwide, and the 2010 Global Burden of disease study showed that diabetes was the ninth leading cause of death in the world **(international Diabetes Federation, IDF, 2011 and Lozano et al., 2012).** In Palestine, there is under diagnosis and under reporting of diabetes mellitus. This is due to lack of proper hospital and clinic recording system **(Ministry of Health, 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 9.5 per 100,000 population in the year 2013 **(MOH, 2014),** and this figure raised to 11.2 per 100,000 population in the year 2014 **(MOH, 2014).** In Gaza strip the incidence rate of diabetes mellitus 15.4 per 100,000 of population in the year 2014. 14.2 per 100,000 of population for diabetes type 2 and 1.2 per 100,000 of population for diabetes type1 **(MOH, 2014).**

2.3.3 Carbohydrate metabolism in type 1 diabetes

Uncontrolled type 1 diabetes leads to increased hepatic glucose output. First, liver glycogen stores are mobilized then hepatic gluconeogenesis is used to produce glucose. Insulin deficiency also impairs non hepatic tissue utilization of glucose. In particular in adipose tissue and skeletal muscle, insulin stimulates glucose up take. This is accomplished by insulin mediated movement of glucose transporters proteins to the plasma membrane of these tissues. Reduced glucose uptake by peripheral tissues in turn leads to a reduced rate of glucose metabolism. In addition, the level of hepatic glucokinase is regulated by insulin. Therefore, a reduced rate of glucose phosphorylation in hepatocytes leads to increased delivery to the blood. The

combination of increased hepatic glucose production and reduced peripheral tissues metabolism leads to elevated plasma glucose level **(Ozougwu et al., 2013)**. When the capacity of the kidneys to absorb glucose is suppressed, glycosuria ensues **(Maori et al., 2014)**. Glucose is an osmotic diuretic and an increase in renal loss of glucose is accompanied by loss of water and electrolyte. The result of the loss of water leads to the activation of the thirst mechanism (polydipsia). The negative caloric balance, which results from the glycosuria and tissue catabolism leads to an increase in appetite and food intake that is polyphagia **(Raju and Raju, 2010).**

2.3.4 Lipid metabolism in type 1 diabetes

In uncontrolled type 1 diabetes where insulin is lacked there is a rapid mobilization of triglycerides leading to increased level of plasma free fatty acids. The free fatty acids are taken up by numerous tissues (except the brain) and metabolized to provide energy. In the absence of insulin , malonyl COA levels fall, and transport of fatty acyl-COA into the mitochondria increases. Mitochondrial oxidation of fatty acids generates acetyl COA that can be further oxidized in the tricarboxylic acid cycle (TCA cycle). However, in hepatocytes the majority of the acetyl COA is not oxidized by the TCA cycle but is metabolized into the ketone bodies (acetoacetate and b hydroxybutyrate). These ketone bodies are used for energy production by the brain, heart and skeletal muscle (**Dashty, 2014)**. In type 1 diabetes, the increased availability of free fatty acids and ketone bodies exacerbates the reduced utilization of glucose, furthering the ensuing hyperglycemia. Production of ketone bodies in excess of the body's ability to utilize them leads to ketoacidosis **(Sumathi and Kalaiselvi, 2014).** A spontaneous breakdown product of acetoacetate is the acetone that is exhaled by the lungs, which gives a distinctive odor to the breath. Normally, plasma triglycerides are acted upon by lipoprotein (LPL) that requires insulin. LPL is a membrane bound enzyme on the surface of the endothelial cells lining the vessels, which allows fatty acids to be taken from circulating triglycerides for storage in adipocytes **(Raju and Raju, 2010).** The absence of insulin results in hypertriglyceridemia.

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 and Worsley, 1993)**. The bacterium displays remarkable motility in viscous solutions, and the flagella play a central role in this motility **(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 and Chu et al., 2010)**.

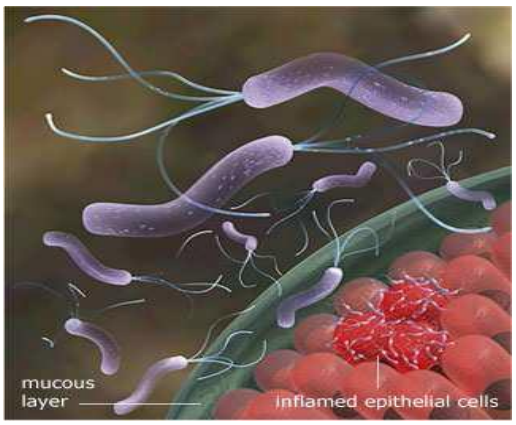


Figure 2.1 *Helicobacter pylori*. The curved bacillus with unipolar flagella is visualized by a scanning electron microscope **(Charles and Janeway, 2005).**

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 (2007)** and **Abu Jabal (2012)** showed that the rate of *H. pylori* infection in Gaza strip was 48.3% and 70.5%, respectively. In a recent study focused on *H. pylori* infection and type 2 diabetes in Gaza strip, **Yassin et al. (2014)** reported that the prevalence of *H. pylori* among type 2 diabetic patients was 72.2 % compared to 36.7% in controls.

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)**.

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 the study. In diabetic patients, the frequency of *H. pylori* infection was higher than in control subjects in nearly all age groups. In this context, **De Luis et al. (1997)** found that the seroprevalence of *H. pylori* among type 1 diabetic patients aged less than 24 years was significantly higher than among control subjects. In addition, **Salardi et al. (1999)** concluded that diabetic children show an *H. pylori* seroprevalence tendentially higher than that of controls of the same age.

Arslan et al. (2000) assessed the seroprevalence of *H. pylori* in 88 type 1 diabetic children and 42 healthy controls using a serological test for *H. pylori* (anti- *H. pylori*

IgG with ELISA). Anti- *H. pylori* IgG was positive in 49/88 (55.6%) of diabetics and 13/42 (30.9%) of controls ($p<0.01$). Diabetic children were divided into two groups according to *H. pylori* status: *H. pylori* (+) and *H. pylori* (-). The two groups were compared for age, gender, duration of diabetes, diabetic control (HbA1c), height and gastric emptying time. Seroprevalence of *H. pylori* was higher in type 1 diabetic patients than in healthy controls. Duration of diabetes was the only factor which correlated significantly with *H. pylori* status.

The incidence of *H. pylori* re-infection after a successful therapy in type 1 diabetic patients was investigated **(Ojetti et al., 2002)**. A total of 74 subjects previously infected by *H. pylori* were enrolled including 34 type 1 diabetic subjects and 40 nondiabetic controls. They found a significantly higher incidence of *H. pylori* re-infection in type 1 diabetic patients compared with nondiabetic controls. In particular, 13 of 34 (38%) type 1 diabetic patients compared with 2 of 40 (5%) control subjects were re-infected with *H. pylori* one year after successful eradication ($P<0.001$). In addition, **Shamsi et al. (2004)** found that sera were positive for anti-*H. pylori* in 22.7% of type 1 diabetics versus 17.3% in controls.

Toporowska et al. (2007) reported high prevalence of *Helicobacter pylori* infection among type 1 diabetic patients. HbA1c concentration was significantly higher in patients with *Helicobacter pylori* infection (7.87+/-1.51 vs. 7.17+/-1.46%; $p<0.05$). In this context, **Al-Bayati and Yas (2010)** indicated that *H. pylori* infection is more common in type 1 and type 2 diabetic patients particularly those with poor glycemic control.

In his study entitled "Helicobacter pylori infection might be responsible for the interconnection between type 1 diabetes and autoimmune thyroiditis", **El-Eshmawy et al. (2011)** measured Anti- *H. pylori* IgG and IgA in 162 euthyroid patients with type 1 diabetes and 80 healthy controls matched for age, sex and socioeconomic status. Seroprevalence of *H. pylori* was significantly higher in patients with type 1 diabetes than in healthy controls; 79% vs. 51.2%, $p<0.001$. Anti *H. pylori* IgG was positive in 61.1% of patients with type 1 diabetes and 30% of controls, $p<0.001$, anti-*H. pylori* IgA was positive in 74% of patients with type 1 diabetes and 32.5% of controls, $p<0.001$.

Candelli et al. (2012) evaluated the reinfection rate of *H. pylori* three years after a standard eradicating treatment in 69 type 1 diabetic patients and 99 controls. The prevalence of *H. pylori* infection was higher in type 1 diabetic patients than in controls (17/69, 24% vs 7/99, 7%, P<0.005, OR: 1.96 [1.67-11.04]) of similar age, gender and socioeconomically status after three years of follow-up. The reinfection rate was higher in type 1 diabetic patients than in controls. In addition, **Talebi-Taher et al. (2012)** found that prevalence of *H. pylori* infection was significantly higher in diabetics than in nondiabetics (P=0.001).

The prevalence of *H. pylori* among 50 type1 and type 2 diabetic patients compared to 50 nondiabetics was determined **(Mohamady et al., 2013**). Positive cases for *H. pylori* infection by (HpSAg) test was 61.1% in type 1 diabetic patients and 65.6% in type 2 diabetic patients compared to 50% of the non-diabetic group. Glycosylated Hb was higher among positive cases. In their meta-analysis of 39 studies involving more than 20,000 participants, **Wang et al. (2013)** reported that *H. pylori* was associated with an increased risk of type 1 and type 2 diabetes mellitus. In addition, **Zekry et al. (2013)** pointed out that the seroprevalence rate of *H. pylori* was significantly higher in type 1 diabetic patients than controls, and the duration of diabetes was significantly longer in *H. pylori*-positive patients with higher levels of HbA1c and insulin requirement.

Fayed et al. (2014) evaluated *H. pylori* infection and virulent strain in 53 type 1 diabetic children. Fifty three normal volunteers were included as controls. All studied children were subjected to assessment of HbA1, Anti- *H. pylori* antibodies (IgA, IgG, IgM), Anti-cytotoxin associated gene A antibodies (Anti Cag A IgG). Anti *H. pylori* antibodies IgA, IgG, total antibodies and anti Cag A IgG were significantly higher in diabetics. Diabetic patients with positive anti Cag A IgG had a lower age of onset of diabetes, higher age of patients, body mass index (BMI) and HbA1.

Chapter 3

Materials and Methods

3.1 Study design

The present study is a case control-study. Case-control studies are often used to identify factors that may contribute to a medical condition by comparing subjects who have that condition/disease (the "cases") with subjects who do not have the condition/disease but are otherwise similar (the "controls"). Case-control studies are quick, widely used, relatively inexpensive to implement, require comparatively fewer subjects, and allow for multiple exposures or risk factors to be assessed for one outcome **(Mann, 2003 and Song and Chung, 2010).**

3.2 Study population

The study population comprised type 1 diabetic children aged up to 18 years attending various diabetic Units at Al-Ranteesy Hospital and Palestinian Medical Relief Center in Gaza strip. Control group was non-diabetic apparently healthy children.

3.3 Sample size and sampling

Non probability accidental sample of type 1 diabetic children, previously diagnosed according to the World Health Organization diagnostic criteria for diabetes **(WHO, 2006)**, were selected as cases from Diabetic Units at Al-Ranteesy Hospital and Palestinian Medical Relief Center in Gaza strip. Controls were apparently healthy non diabetic children selected from the general population. Cases and controls were age and gender matched. The sample size calculations were based on the formula for case-control studies. EPI-INFO statistical package version 3.5.1 was used with 95% CI, 80% power and 50% proportion as conservative and OR > 2. The sample size in case of 1:1 ratio of case control was found to be 54:54. For a no-response expectation, the sample size was increased to 60 patients. The controls also consisted of 60 non diabetic individuals.

3.4 Exclusion criteria

- Cases and controls whose aged above 18 years old.
- Type 2 diabetic patients.
- Patients with other chronic diseases.
- Patients who take hormone replacement therapy or corticosteroid therapy.

3.5 Ethical Consideration

An official letter of request was sent from the Palestinian Ministry of Health to Medical Center Administrations in different Gaza hospitals to facilitate the conduction of the study ***(*Annex 1*).*** The participants were given a full explanation about the purpose of the study and assurance about the confidentiality of the information and the participation was optional**.** 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 Limitation of the study

1. The number of patients who frequently visiting the diabetic clinics was relatively low.

2. Many patients and their family refused to supply blood sample.

3.7 Data collection

3.7.1 Questionnaire interview

A meeting interview was used for filling in a questionnaire which designed for matching the study need for both cases and controls ***(Annex 2)***. All interviews were conducted face to face by the researcher herself. During the survey the interviewer explained any of the questions that were not clear. The questionnaire was based on recent studies conducted on diabetes melitus in Gaza Strip with some modifications **(Elhamalawi, 2015)**. 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 endocrinology, epidemiology, public health and biochemistry. The questionnaire was piloted with 8 patients not included in the study. The questionnaire included questions on the socioeconomic data of the study population (Age, gender, family income/month and family history of diabetes), diet and compliance of medication, and clinical data including duration of diabetes, self-reported complications, gastritis and peptic ulcer.

3.7.2 Body mass index

Body mass index was calculated as the ratio of body weight in Kg/height in square meter. Patients were asked to remove heavy clothes and shoses before measurement of weight and height. Medical balance (Seca Model 762, Germany) was used for weight measurement. children with BMI=15.0-20.0Kg/m^2 were considered to have normal weight, and children with BMI≥30.0 were classified obese **(CDC, 2000)**.

3.7.3 Specimen collection and biochemical analysis

Twelve hours fasting overnight venous blood samples were collected from 60 type 1 diabetic children and 60 healthy non diabetic controls. Blood samples (6 ml each) were drawn by a well-trained nurse into vacutainer and plastic tubes. 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 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. *H. pylori* IgG was determined in serum by ELISA kit.

3.8 Biochemical analysis

3.8.1 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:

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

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

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

$\text{Ab}_{(\text{X-}H.\ pylori)}$-${}^{\text{Btn}}\text{Ag}_{(H.\ pylori)}$ = 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:

$\text{h-Ab}_{(\text{X-}H.\ pylori)}$-${}^{\text{Btn}}\text{Ag}_{(H.\ pylori)}$+$\text{Streptavidin}_{\text{CW}}$ $\longleftrightarrow$ immobilized complex (IC)

$\text{Streptavidin}_{\text{CW}}$ = 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.

$\text{IC}_{(\text{h- IgG,M or A})} + {}^{\text{ENZ}}\text{Ab}_{(\text{X-h-IgG, M or A})} \Rightarrow {}^{\text{ENZ}}\text{Ab}_{(\text{X-h-IgG, M or A})} - \text{IC}_{(\text{h- IgG, M or A})}$

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

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

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

Reagents

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°C. 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.

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°C.

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°C.

E. Serum Diluent – 20ml

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

F. Wash Solution Concentrate – 20ml

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

G. Substrate A – 7ml/vial

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

H. Substrate B – 7ml/vial

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

I. Stop Solution – 8ml/vial

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

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°C).

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. Add 100µl of Working Substrate Solution to all wells.

11. Incubate at room temperature for 15 minutes.

12. Add 50µl of stop solution to each well and swirl the microplate gently for 15-20 seconds to mix.

13. 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.

3.8.2 Determination of glycated hemoglobin in whole blood

Glycated hemoglobin was determined by the colorimetric determination of glycated hemoglobin in whole blood using Stanbio Kit, Texas-USA **(Trivelli 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 Contains potassium cyanide 10 mmol/L and surfactants.
Glycated hemoglobin Standard (Lyophilized) (1 vial) 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°C) to complete hemolysis.

Glycated hemoglobin separation and assay

1. Label resin tubes Standard (S), Unknown (U) and Control (C).
2. Pipette I00 µl of the prepared hemolysate into appropriately labeled resin tube.
3. Position a resin separator in the tube so rubber sleeve is approximately 1-2 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:

$$(R) = Agly / Atot$$

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

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

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

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

3.8.3 Determination of serum glucose

Serum glucose 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) Phenol 4-Aminoantipyrine Glucose oxidase (GOD) Peroxidase (POD)	250 mmol/l 5 mmol/l 0.5 mmol/l ≥ 15 ku/l ≥ 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

3.8.4 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

3.8.5 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.

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

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

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

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

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

Reference value

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

3.8.6 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.

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

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

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

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

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/I
Peroxidase (POD)	≥ 2 KU/I
Lipoprotein lipase (LPL)	≥ 2 KU/I
4-Aminoantipyrine	0.5 mmol/l
Glycerol-3-phosphate-oxidase (GPO)	≥ 0.5 KU/I
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

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

Reference value

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

3.8.7 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 Phosphotungstic acid	1.4 mmol/l 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

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

Reference value

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

3.8.8 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

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

3.8.9 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

$$\text{L- Asprtate + 2-Oxoglutarate} \xrightarrow[\text{MDH}]{\text{ASAT}} \text{L- Glutamate + Oxalacetate}$$

Oxalacetate + NADH + H^+ ⟷ L - Malate + NAD^+

Reagents

Reagent	Components	Concentrations
Reagent 1	TRIS pH 7.65 L- Aspartate MDH (Malate dehydrogenase) LDH (lactate dehydrogenase)	80 mmol/l 240 mmol/l ≥ 600 U/l ≥ 900 U/l
Reagent 2	2-Oxoglutarate NADH	12 mmol/l 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 ^oC & 5 days at 15-25 ^oC. 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

3.8.10 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 $\xleftrightarrow{\text{ALAT}}$ L- Glutarate + Pyruvate

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

Reagents

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

3.8.11 Determination of serum urea

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

Principle

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_4 + H2CO$$

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

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

ΔA = (A1 –A2) sample or standard

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

Reference value

(PCLTG, 2005)

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

3.8.12 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 ⟶ 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

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

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 F	0.6 - 1.2 mg/dl 0.5 -1.1 mg/dl

3.9 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), hematocrit (Hct) and platelet (PLT) content.

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

The present study is a case control design. The study population comprised 60 type 1 diabetic children who represent cases (30 males and 30 females) and 60 apparently healthy controls (30 males and 30 females). Cases and controls are age matched (≤18 years). Table 4.1 illustrates sociodeogrphic data of the study population. Age classification showed that 5 (8.3%) cases and 5 (8.3%) controls were <6 years old. Age group 6-12 years comprised 30 (50.0%) cases and 31 (51.7%) controls. Cases and controls aged >12 years old were 25 (41.7%) and 24 (40.0%), respectively. The difference between controls and cases in term of age distribution was not significant (χ^2=0.037 P=0.982). The mean ages of cases and controls were 11.1±3.9 and 11.2±3.8 years old. The independent sample t-test also showed no significant difference between mean ages of cases and controls (t=0.118, P=0.907). Family income\month showed that the number of cases with high income was higher than that of controls. The difference between the two groups was significant ($\chi^2_{(corrected)}$=6.617, P=0.037), implying that family income is associated with diabetes. Regarding family history of diabetes, 37 (61.7%) cases and 22 (36.7%) controls reported that they have family history of the disease, whereas 23 (38.3%) cases and 38 (63.3%) controls did not. The difference between the two groups was significant (χ^2=7.502, P=0.006) indicating that family history is associated with diabetes.

Table 4.1 Sociodemographic data of the study population

Sociodemographic data	Cases (n=60)		Controls (n=60)		Test		P-value
	n	%	n	%			
Age (Year)							
<6	5	8.3	5	8.3	χ^2	0.037	0.982
6-12	30	50.0	31	51.7			
>12	25	41.7	24	40.0			
Mean±SD	11.1±3.9		11.2±3.8		t	0.118	0.907
Gender							
Male	30	50.0	30	50.05	χ^2	0.000	1.000
Female	30	50.0	30	0.0			
Family income/month (NIS)*							
<1000	27	45.0	18	30.0	χ^2	6.617	0.037*
1000-2000	24	40.0	39	65.0			
>2000	9	15.0	3	5.0			
Family history of diabetes							
Yes	37	61.7	22	36.7	χ^2	7.502	0.006
No	23	38.3	38	63.3			

*P-value of χ^2 (corrected) test. P<0.05: Significant, P>0.05: Not significant.

4.2. Diet and compliance of medication among cases

Table 4.2 illustrates diet and compliance of medication among cases. Fifteen cases (25.0%) were reported to be on diet whereas 45 (75.0%) were not. However, the majority of cases 57 (95.0%) were found to be compliance of medication.

Table 4.2 Diet and compliance of medication among cases

Item	n	% of cases
Diet		
Yes	15	25.0
No	45	75.0
Compliance of medication		
Yes	57	95.0
No	3	5.0

n: number of diabetic patients.

4.3. Distribution of diabetic patients by the duration of the disease

Distribution of diabetic patients by disease duration is demonstrated in Table 4.3. Patients with diabetes since less than 5 years were 40 (66.7%), whereas those with diabetic duration of 5-10 years were 17 (28.3%). The rest of patients 3 (5.0%) had diabetes for more than 10 years.

Table 4.3. Distribution of diabetic patients (n=60) by the duration of the disease.

Duration of diabetes (Year)	n	%
< 5	40	66.7
5-10	17	28.3
>10	3	5.0

n: number of diabetic patients.

4.4 Self-reported complications of the study population

Table 4.4. provides the main self-reported complications among the study population. Although the percentage of retinopathy was higher in cases than controls, the difference between the two groups was not significant (10.0% versus 3.3%, $\chi^2_{(corrected)}$=1.205, P=0.272). However, significant difference was found for neuropathy with higher percentage among cases (18.3% versus 1.7%, $\chi^2_{(corrected)}$=7.500, P=0.006). Cardiovascular disease was not self-reported among cases and controls.

Table 4.4 Self-reported complications among the study population.

Self-reported complication	cases (n=60)		controls (n=60)		χ^2	P-value*
	n	%	n	%		
Retinopathy						
Yes	6	10.0	2	3.3	1.205	0.272
No	54	90.0	58	96.7		
Neuropathy						
Yes	11	18.3	1	1.7	7.500	0.006
No	49	81.7	59	98.3		

*P-value of χ^2 (corrected) test. P<0.05: Significant, P>0.05: Not significant.

4.5 Gastritis and peptic ulcer among the study population

Table 4.5 demonstrates the distribution of gastritis and peptic ulcer among the study population. Gastritis was found in 8 (13.3%) cases versus one control (1.7%), with significant difference between the two group (χ^2 (corrected)=4.324, P=0.038). In addition, peptic ulcer was found in 3 (5.0%) cases compared to 1 (1.7%) controls (χ^2 (corrected)=0.259, P=0.611).

Table 4.5 Gastritis and peptic ulcer among the study population.

Item	cases (n=60)		controls (n=60)		χ^2	P-value*
	n	%	n	%		
Gastritis						
Yes	8	13.3	1	1.7	4.324	0.038
No	52	86.7	59	98.3		
Peptic ulcer						
Yes	3	5.0	1	1.7	0.259	0.611
No	57	95.0	59	98.3		

*P-value of χ^2 (corrected) test. P<0.05: Significant, P>0.05: Not significant.

4.6 Body mass index of the study population

The BMI of the study population is pointed out in Table 4.6. The mean weight of cases was 37.9±16.1 Kg compared to 36.7±15.4 Kg of controls (% difference = 3.2% and P=0.685). In addition, the mean height of cases was 1.39±0.21 m compared to 1.42±0.22 m of controls (% difference=2.1 and P=0.486). Therefore, BMI was

significantly increased in cases compared to controls (18.6±3.8 versus 17.3±3.3, % difference=7.2% and P=0.047).

Table 4.6. Body mass index of the study population.

Anthropometric measurement	Case (n=60) Mean ±SD	control (n=60) Mean ±SD	% difference	t	P-value
Weight (kg)* *(min-max)*	37.9±16.1 *(13-77)*	36.7±15.4 *(13-70)*	3.2	0.406	0.685
Height (m)** *(min-max)*	1.39±0.21 *(84-174)*	1.42±0.22 *(98-175)*	-2.1	0.699	0.486
BMI*** *(min-max)*	18.6±3.8 *(11.8-29.3)*	17.3±3.3 *(12.2-26)*	-7.2	2.007	0.047

*Kg: kilogram, ** m: meter. ***BMI: Body Mass Index (Kg/m^2): Normal=15.0-20.0, Obese≥23.0 (CDC, 2000). P<0.05: Significant, P>0.05: Not significant.

4.7 Glycated hemoglobin, glucose and insulin levels of the study population

As indicated in Table 4.7, the mean levels of blood HbA1c was significantly higher in cases than controls (9.8±1.8% versus 5.0±0.5%, % differences=64.8 and P=0.001). Similarly, the levels of serum glucose and insulin were significantly higher in cases (169.6±111.9 mg/dl and 26.7±17.0 mlU/ml) compared to controls (77.7±7.0 mg/dl and 10.6±4.7 mlU/ml) with % differnces= 74.3 and 86.3 and P=0.001, respectively.

Table 4.7. Blood HbA1c, glucose and insulin levels among the study population.

Parameter	Case (n=60) Mean ±SD	Control (n=60) Mean ±SD	% difference	t	P-value
HbA1c (%)* *(min-max)*	9.8±1.8 *(6.2-13.7)*	5.0±0.5 *(4.1-5.9)*	-64.8	20.315	0.001
glucose (mg/dl) *(min-max)*	169.6±111.9 *(62-530)*	77.7±7.0 *(68-96)*	-74.3	6.355	0.001
Insulin (mlU/ml) *(min –max)*	26.7±17.0 *(4.6-67.7)*	10.6 ± 4.7 *(2.3-28)*	-86.3	7.082	0.001

*HbA1c: Glycated hemoglobin. P<0.05: Significant.

4.8 Serum lipid profile of the study population

Table 4.8 illustrates serum lipid profile of the study population including cholesterol, triglycerides, LDL-C and HDL-C. The mean levels of cholesterol, triglycerides and LDL-C showed no significant increases in cases with respect to controls (P>0.05). However, HDL-C displays no significant decrease in cases compered to controls (P>0.05).

Table 4.8. Lipid profile of the study population.

Lipid profile (mg/dl)	Case (n=60) Mean ±SD	Control (n=60) Mean ±SD	% difference	t	P-value
Cholesterol *(min-max)*	171.3±26.9 *(140-288)*	164.0±18.4 *(125-210)*	-4.5	1.681	0.093
Triglycerides *(min-max)*	121.8±37.7 *(73-248)*	110.2±28.3 *(71-192)*	-10	1.894	0.064
LDL-C * *(min-max)*	95.0±25.5 *(61-199)*	88.3±18.4 *(63-148)*	-7.3	1.755	0.082
HDL-C ** *(min-max)*	53.7±3.3 *(41-59)*	54.6±2.5 *(50-60)*	1.6	1.758	0.080

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

4.9 Liver and kidney functions of the study population

The activities of serum AST and ALT as marker enzymes of liver function as well as the concentrations of serum urea and creatinine as indicators of kidney function are pointed out in Table 4.9. There were significant elevations in AST and ALT activities in cases compared to controls (32.1±7.8 and 26.5±12.4 U/L *versus* 22.0±10.5 and 19.5±7.5 U/L, % difference=27.5 and 30.4 and P=0.000, respectively). On the other hand, urea and creatinine concentrations showed no significant difference between cases and controls (P> 0.05).

Table 4.9. Liver and kidney functions of the study population.

Parameter	Cases (n=60) Mean ±SD	controls (n=60) Mean ±SD	% Difference	t	P-value
AST (U/L) *(min-max)*	32.1±7.8 *(18-62)*	22.0± 10.5 *(10 -58)*	27.5-	6.005	0.000
ALT (U/L) *(min-max)*	26.5±12.4 *(12-85)*	19.5±7.5 *(10 -49)*	30.4-	3.754	0.000
Urea (mg/dl) *(min-max)*	26.6±4.5 *(21 -39)*	25.3± 3.9 *(20-36)*	-5.9	1.701	0.092
Creatinine (mg/dl) *(min-max)*	0.76±0.07 *(0.61-0.99)*	0.74±0.08 *(0.48-0.90)*	-2.6	1.617	0.108

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase. P<0.05: Significant, P>0.05: Not significant.

4.10 Blood parameters of the study population

Table 4.10 illustrates blood parameters of the study population. White blood cell count was increased in cases compared to controls (7.5±2.4 versus 6.7±1.8, % differences 11.3, t=2.200 and P=0.030). Similarly, blood platetes were increased in cases but such increase was not significant (319.2±90.6 versus 302.0±76.5, % differences 5.5, t=1.113 and P=0.268). On the other hand, RBC count and hemoglobin content showed significant decreases in cases than controls (4.5±0.4and 11.7±1.2 versus 4.7±0.5 and 12.4±1.3, % differences 4.7 and 5.8, P=0.016 and 0.005 respectively. Hematocrit showed no significant differences between cases and controls (P=0.110).

Table 4.10 Blood parameters of the study population.

Blood parameter	Cases (n=60) Mean ±SD	controls (n=60) Mean ±SD	% Difference	t	P-value
WBC $\times 10^9$/L *(min-max)*	7.5±2.4 *(4.3-17.4)*	6.7± 1.8 *(4.1-12.9)*	11.3	2.200	0.030
RBCs $\times 10^{12}$/L *(min-max)*	4.5±0.4 *(3.7-5.8)*	4.7± 0.5 *(2.4-5.6)*	4.7	2.447	0.016
Hb (g/dl) *(min-max)*	11.7±1.2 *(9.2-14.9)*	12.4±1.3 *(7.1-15.7)*	5.8	2.833	0.005
Hct(%) *(min-max)*	37.8±3.8 (22-46.6)	38.9±4.0 (30.6-49.6)	3.8	1.609	0.110
PLT $\times 10^9$/L *(min-max)*	319.2±90.6 *(181-602)*	302.0± 76.5 *(180-586)*	5.5	1.113	0.268

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

4.11 Distribution of *Helicobacter pylori* IgG among the study population

Distribution of *H. pylori* IgG among the study population is presented in Table 4.11 and Figure 4.1. Twenty four (40.0%) cases were positive for *H. pylori* IgG compared to 13 (21.7%) controls. The difference between the two groups was significant (χ^2=4.728, P=0.030) with higher distribution of *H. pylori* IgG among cases.

Table 4.11 Distribution of *Helicobacter pylori* IgG among the study population.

***Helicobacter pylori* IgG**	**Cases (n=60)**	**Controls (n=60)**	**χ^2**	**P-value**
	n (%)	**n (%)**		
***Helicobacter pylori* IgG** Positive Negative	24 (40.0) 36 (60.0)	13 (21.7) 47 (78.3)	4.728	0.030

P<0.05: Significant. Presence of *H. pylori* IgG confirmed by IgG ≥20U/ml.

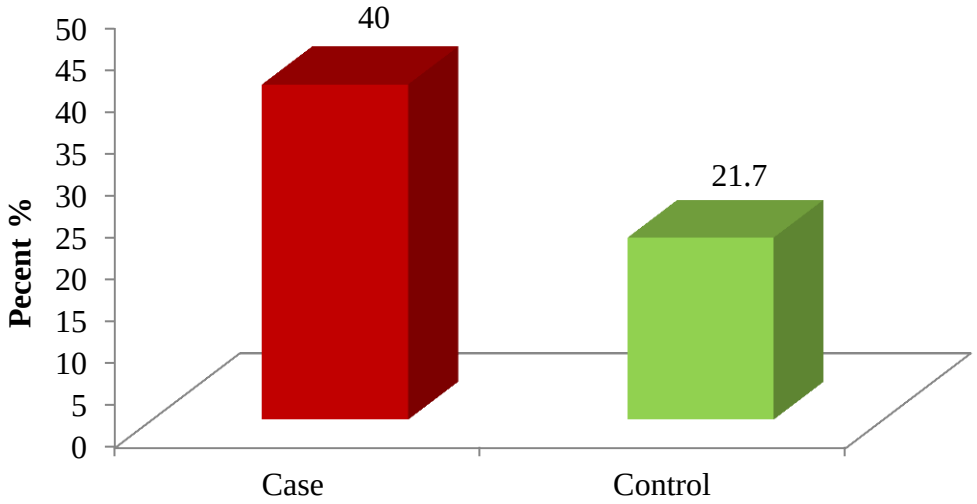


Figure 4.1. Distribution of Helicobacter pylori infection among the study population

4.12 Relations of *Helicobacter pylori* to different parameters

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

As indicated in Table 4.12, *H. pylori* positive cases showed significant increase in BMI compered to negative cases (21.0±3.8 *versus* 17.8±3.5, P=0.003).

Table 4.12 *Helicobacter pylori* in relation to body mass index of cases (n=60).

Parameter	*Helicobacter pylori*	Cases (n=60)	Mean ±SD	t	P-value
BMI	Positive	24	21.0±3.8	3.046	0.003
	Negative	36	17.8±3.5		

BMI: Body Mass Index: Normal=15.0-20.0, Obese≥23.0 (CDC, 2000). P<0.05: Significant.

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

Table 4.13 illustrated the relationship between *H. pylori* and blood HbA1c and serum glucose and insulin of cases. The mean level of blood HbA1c in positive cases was significantly higher than that in negative cases (10.5±1.7 *versus* 9.5±1.7, P=0.046). Similarly, serum glucose and insulin levels were significantly higher in positive than nigative cases (233.8±145.2 and 34.2±21.7 *versus* 146.3±87.9 and 23.9±14.2, P=0.006 and 0.037, respectively).

Table 4.13 *Helicobacter pylori* in relation to glycated hemoglobin, glucose and insulin of cases.

Parameter	*Helicobacter pylori*	Cases (n=60)	Mean±SD	t	P-value
HbA1c (%)	Positive	24	10.5±1.7	2.041	0.046
	Negative	36	9.5±1.7		
glucose (mg/dl)	Positive	24	233.8±145.2	2.836	0.006
	Negative	36	146.3±87.9		
Insulin (□lU /ml)	Positive	24	34.2 ± 21.7	2.132	0.037
	Negative	36	23.9±14.2		

HbA1c: Glycated hemoglobin. P<0.05: Significant.

4.12.3 *Helicobacter pylori* in relation to lipid profile of cases

Helicobacter pylori in relation to lipid profile including cholesterol, triglycerides, LDL-C, and HDL-C is presented in Table 4.14. The mean levels of cholesterol, triglycerides and LDL-C in positive cases were significantly higher than that in negative cases (186.2±35.0, 135.5±44.2 and 107.9±32.9 mg/dl, *versus* 157.8±19.4, 99.5±29.4 and 83.4 ±18.6 mg/dl, P=0.001, P=0.001 and P=0.001, respectivily). On the other hand the mean level of HDL-C was significantly lower in positive compared to negative cases (51.3 ±3.7 *versus* 54.6±2.6 mg/dl, P=0.001).

Table 4.14 *Helicobacter pylori* in relation to lipid profile of cases.

Lipid profile (mg/dl)	*Helicobacter pylori*	Cases (n=60)	Mean ±SD	t	P-value
Cholesterol	Positive Negative	24 36	186.2±35.0 157.8±19.4	3.985	0.001
Triglyceride	Positive Negative	24 36	135.5±44.2 99.5±29.4	3.643	0.001
LDL-C*	Positive Negative	24 36	107.9±32.9 83.4 ±18.6	3.623	0.001
HDL-C**	Positive Negative	24 36	51.3 ±3.7 54.6±2.6	3.891	0.001

*LDL-C: Low density lipoprotein cholesterol, **HDL-C: High density lipoprotein cholesterol. P<0.05: Significant.

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

Table 4.15 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 (29.1±8.5 versus 24.5±7.3U/L, P=0.045). However AST showed no significant difference between positive and negative cases (33.9 ±9.8 versus 30.4±7.0U/L, P=0.123). In addition urea and creatinine concentrations showed no significant increases in positive compered to negative cases (26.9±4.6 and 0.77±0.06 versus 25.0±3.7 and 0.75±0.07mg/dl, P=0.128 and 0.162, respectively).

Table 4.15 *Helicobacter pylori* in relation to liver and kidney function of cases.

Parameter	*Helicobacter pylori*	Cases (n=60)	Mean ±SD	t	P-value
AST (U/L)	Positive Negative	24 36	33.9 ±9.8 30.4±7.0	1.554	0.123
ALT (U/L)	Positive Negative	24 36	29.1±8.5 24.5±7.3	2.028	0.045
Urea (mg/dl)	Positive Negative	24 36	26.9±4.6 25.0±3.7	1.527	0.128
Creatinine (mg/dl)	Positive Negative	24 36	0.77±0.06 0.75±0.07	1.447	0.162

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase. P<0.05: Significant, P>0.05: Not significant.

4.12.5 *Helicobacter pylori* in relation to blood parameters of cases

The relationship between *H. pylori* and blood parameters including WBC, RBC, Hb, Hct and PLT is presented in Table 4.16. The WBC count was significantly higher in positive compared to negative cases (7.8±1.5 vs 6.7±1.9 $\times 10^9$/L, P=0.041). Conversely, RBC and Hb were significantly decrease in positive cases than negative cases (4.6±0.5$\times 10^{12}$/L and 11.9±1.1 g/dl vs 4.9±0.4$\times 10^{12}$/L and 13.0±1.4 g/dl, P=0.023 and 0.012, respectively). However, there were no significant differences in Hct and PLT between positive cases and negative cases (P>0.05).

Table 4.16 *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 Negative	24 36	7.8±1.5 6.7±1.9	2.088	0.041
RBCs $\times 10^{12}$/L	Positive Negative	24 36	4.6±0.5 4.9±0.4	2.343	0.023
Hb (g/dl)	Positive Negative	24 36	11.9±1.1 13.0±1.4	2.497	0.012
Hct(%)	Positive Negative	24 36	37.4±4.1 38.8±2.9	1.525	0.135
PLT $\times 10^{12}$/L	Positive Negative	24 36	326.4±98.0 294.3±61.2	1.334	0.192

WBC: White blood cell, RBC: Red blood cell, Hb: Hemoglobin, Hct: Hematocrit, PLT: Platelet. P<0.05: Significant, P>0.05: Not significant.

Chapter 5

Discussion

Type 1 diabetes mellitus is the most common metabolic disease among children, adolescents and young adults and its incidence rate is still rapidly increasing particularly in developing countries. Annually over 17 new cases of type 1 diabetes were identified per 100,000 children globally and there were about half million of diabetes-related deaths worldwide **(Patterson et al., 2009 and International Diabetes Federation, IDF, 2011).** In Palestine the mortality rate of diabetes mellitus constitutes 11.2 per 100,000 populations in the year 2014 **(MOH, 2014).** Despite that, there is a lack of a proper hospital and clinic recording system of diabetes mellitus in Palestine. In addition, most research in Gaza strip was focused on type 2 diabetes rather than type 1. Furthermore, to our best knowledge there was no previous study linked type 1 diabetes with *H. pylori* in Gaza Strip. Therefore, the present study is the first to assess *H. pylori* infection among type 1 diabetic children in Gaza strip.

5.1 Sociodemographic data of the study population

The present results demonstrated that type 1 diabetes was significantly more frequent among children's families with higher income than that with lower income.

In general, socioeconomic status is accepted as a risk factor for type 1 diabetes **(Galler et al., 2011).** Regarding family history of diabetes, the number of cases who reported family history of type 1 diabetes was significantly higher than controls. This implies that family history is associated with type 1 diabetes. Such finding is in agreement with that reported by **Bonifacio et al. (2004); Mehers and Gillespie (2008) and Parkkola et al. 2013)**. In this context, **Alhonen et al. (2011)** concluded that type 1 diabetes and autoimmune diseases not only cluster in the nuclear families of children with type 1 diabetes but are also overrepresented in their extended families.

5.2. Diet, compliance of medication and distribution of diabetic patients by duration of disease

Data presented in this study indicated that three quarters of cases were not on diet. However, the majority of cases were found to be compliance of medication. **Wolpert et al. (2013)** suggested that dietary fat intake is an important nutritional consideration for glycemic control in individuals with type 1 diabetes. In addition, **Rize et al. (2014)** found that more than half of type 1 diabetic patients were non adherent to dietary advice. These findings necessitate launch of awareness programs on the importance of diet in the management of diabetes mellitus. Clinical data showed that almost two-thirds of patients had diabetes since less than 5 years. This implies that type 1 diabetes affected young individuals. **Belle (2011) and Harjutsalo et al. (2013)** reported that type 1 diabetes usually strikes children and young adults, although disease onset can occur at any age.

5.3 Self-reported complications, gastritis and peptic ulcer among the study population

Neuropathy was significantly more frequent among cases compared to controls, whereas retinopathy showed no significant difference between cases and controls. However, cardiovascular disease was not self-reported among cases and controls.

Diabetic complications including neuropathy and retinopathy were reported among type 1 diabetic patients **(Sun et al., 2011 and Cobuz and cobuz, 2012).** Gastritis was significantly more prevalent among cases compared to controls. Similar result was obtained by **Taher et al. (2012)** who found that gastritis was significantly more frequent in diabetic patients compared to non-diabetics. However, no significant difference was found in peptic ulcer between the two groups.

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

The results of this study showed significantly higher positive *H. pylori* infection 24 (40.0%) among diabetic patients compared to controls 13 (21.7%). The approximate two-fold increment in *H. pylori* infection in diabetic patients indicates that *H. pylori* is associated with type 1 diabetes. Higher prevalence of *H. pylori* was found among type

1 diabetics patients compared to non-diabetics **(El-Eshmawy, 2011; Candelli et al., 2012; Zekry et al, 2013 and Fayed et al., 2014)**. *H. pylori* infection was not associated with socio-demografic status, diet and compliance of medication, self-reported complications or gastritis and peptic ulcer.

5.5 Body mass index of the study population

In the preset study BMI was significantly increased in cases compared to controls. Several studies generally indicated that cases with type 1 diabetes had higher body weight and BMI compared with non-diabetics **(Bishop et al., 2014; Kapellen et al., 2014 and Szadkowska et al., 2015).** In particular, **Fayed et al. (2014)** revealed that BMI was significantly increased in children with type 1 diabetes compared to healthy children. When related to *H. pylori* infection, BMI showed significant increase in positive compared to negative cases. Such finding coincides with that obtained by **Fayed et al. (2014)** who reported high prevalence of *H. pylori* infection among type 1 diabetic children with large BMI. However, **Kayar et al. (2015)** showed no significant association of BMI with *H. pylori* infection. The possible explanation of BMI association with *H. pylori* infection that, uncontrolled diabetic patients who constitute three quarters of our sample may had increase in insulin resistance leading to increase in BMI.

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

Data presented in this study pointed out that the mean levels of blood HbA1c, and serum glucose and insulin were significantly higher in cases than in controls. These results are in agreement with that found by **AL-Suhaimi et al. (2012) and Hasan et al. (2013)** in type 1 diabetes children compared to healthy controls. In addition, a comparative study showed significant elevations in the levels of blood glucose and HbA1c in type 1 and type 2 diabetes as compared with normal healthy individuals **(Siva et al., 2012).** 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 play an important role in the

pathogenesis of diabetic complications like retinopathy, nephropathy, and neuropathy **(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 glycemia, 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 and Phillips and Leow, 2014)**. When related to *H. pylori* infection, blood HbA1c, serum glucose and insulin levels showed positive significant correlations with *H. pylori*. These results highlight the interaction between diabetes mellitus and *H. pylori* infection and are in agreement with that previously reported in diabetic children and adolescence **(Fayed et al., 2014 and Kayar et al., 2015).** In addition, **Zekry and EL-Wahid (2013)** elucidated significant positive linear association between *H. pylori* infection, insulin requirements and HbA1c levels in type 1 diabetes mellitus. This association may be explained by the role of *H. pylori* infection in the induction of chronic inflammation and production of cytokines, leading to impaired secretion of insulin, increased anti-insulin activity, and altered carbohydrate metabolism. All these effects translate into decreased glycemic control and increased insulin requirement **(Zekry and EL-Wahid, 2013)**.

5.7 Lipid profile of the study population

Although the mean levels of cholesterol, triglyceride, and LDL-C levels was increased in cases compared with controls, no significant change was found. On the other hand, no significant decrease was found in HDL-C levels between cases and controls. The normal BMI recorded for cases and controls in the present study would expected such result rather than significant elevations of lipid profiles reported in type 1 diabetic patients **(Masram et al., 2012; and Meissner et al., 2014)**. When related to *H. pylori*, cholesterol, triglycerides and LDL-C levels were significantly higher in positive than in negative cases, However, HDL-C level was significantly lower in positive cases. Similar results was previously reported **(Kayar et al., 2015).**

The association between *H. pylori* infection and lipid profile may be explained on the basis that *H. pylori* trigger high amounts of interleukin- 6 and tumor necrosis factor-α. An increase in these cytokines may 1) cause endothelial dysfunction and insulin resistance, 2) increase the production of hepatic gluconeogenesis and triglycerides and modify the lipid levels by inhibiting the lipoprotein lipase activity and activating hepatic lipogenesis, and 3) may cause inflammation secondary to *H. pylori* triggers the peroxidation of membrane lipids **(Kumaran et al., 2014 and Kayar et al., 2015).**

5.8 Liver and kidney functions of the study population

The mean activates of AST and ALT were significantly elevated in cases compared to controls, whereas there were no significant differences in urea and creatinine levels between cases and controls. These results are in accordance with other studies **(West et al., 2006; Leeds et al., 2009; Siva et al., 2012 and Choudhary et al., 2014)**. Elevation of transaminases as biomarkers for liver function 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 diabetes include oxidantive stress from reactive lipid peroxidation, peroxisomal beta-oxidation, and recruited inflammatory cells **(Kayar et al., 2015).** When related to *H. pylori* infection, only ALT was found to be significantly higher in positive compared to negative cases. This means that ALT activity is associated with *H. pylori* infection. In this context, **Takuma (2011)** demonstrated that *H. pylori* infection was one of the independent risk factors for the development of liver disease, which is manifested in elevation of ALT activity as specific preclinical and clinical biomarker of hepatotoxicity **(Ozer et al., 2008**).

5.9 Hematological profile of the study population

White blood cell count was significantly increased in cases with respect to controls, whereas RBC count and hemoglobin content were significantly decreased in cases than controls. Leukocytosis and anemia were observed in type 1 diabetic patients compared to non-diabetic controls **(Uko et al., 2013)**. In addition, **AL-Muhammadi**

and ALwash (2011) and Kothari and bokariya (2012) reported significant decrease in RBC count and hemoglobin level in young adolescents with type 1 diabetes. Elevation of WBC count observed in type 1 diabetic children could be a result of a stress response **(Uko et al., 2013).** Another possibility of Leukocytosis may reside in increase pro-inflammatory cytokines, which may contribute to leukocytosis **(Kayar et al., 2015).** While several factors contribute to the increased prevalence of anemia in diabetes, the failure of the kidney to increase erythropoietin in response to falling hemoglobin appears to be the dominant factor. Previous report indicated that the occurrence of anaemia in diabetes mellitus is due to the increased non-enzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycemia **(Thomas et al., 2004 and Oyedemi et al., 2011).** It has also been suggested that autonomic degeneration as a result of diabetes may diminish erythropoietin release **(Kothari and bokariya., 2012).** This possibility is in concurrent with our result that neuropathy was significantly higher in type 1 diabetic patients than controls.

When related to *H. pylori*, the WBC count was significantly higher in positive compared to negative cases. Conversely, RBC and Hb were significantly decrease in positive than negative cases. Leukocytosis and anemia were reported in *H. pylori* infection in diabetic patients **(Tanriverd, 2011; Jafarzadeh et al., 2013 and Kayar et al., 2015).** 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- α from epithelial cells in the gastric mucosa **(Iida et al., 2012).** In terms of anemia, association between B12 and iron levels among patients suffering from *H. pylori* infection was addressed **(Affana, 2016).**

Chapter 6

Conclusions & Recommendations

6.1 Conclusions

1. Type 1 diabetes mellitus was more prevalent among families with higher income as well as among individuals with family history of the disease.

2. Three quarters of cases were not on diet. However, the majority of cases were found to be compliance of medication. Almost two-thirds of patients had diabetes since less than 5 years.

3. Neuropathy and gastritis were significantly more frequent 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. There were non significant increase in serum cholesterol, triglycerides and LDL-C levels in cases than controls, whereas HDL-C was not significantly decreased.

7. The activities of serum AST and ALT were significantly elevated in cases in comparison with controls.

8. The WBC count was significantly higher in cases with respect to controls. Conversely, RBC count and Hb content were significantly lower in cases.

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

10. When related to *H. pylori* infection, BMI showed significant increase in positive compared to negative cases.

11. When related to *H. pylori*, blood HbA1c levels, serum glucose and insulin were significantly higher in positive than in negative cases.

12. When related to *H. pylori*, serum cholesterol, triglycerides and LDL-C levels were significantly higher in positive than in negative cases, However, HDL-C level was significantly lower in positive cases.

13. The activity of serum ALT was significantly increased in *H. pylori* positive cases compared to negative cases.

14. The WBC count was singnificantly elevated in *H. pylori* positive cases compared to negative cases, whereas RBC count and Hb content were significantly decreased in positive cases.

6.2 Recommendations

1. Frequent monitoring of *H. pylori* infection among type 1 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 neurological clinics to take early steps to avoid and manage diabetic complications concerning diabetic neuropathy.

4. Further research is highly recommended on *H. pylori* infection among other autoimmune diseases.

References

Abu Jabal, E.A. (2012): The Role of *Helicobacter pylori* Infection, Malnutrition and Insulin Resistance among Type 2 Diabetic Medical Services Patients in the Gaza Strip: A Cross-Sectional Study. Master thesis. Al Azhar University- Gaza.

Abu-Mughesieb, R. (2007): Risk Factors Associated with *Helicobacter pylori* Infection in Gaza, Palestine. Master thesis. Islamic university-Gaza.

Achenbach, P. Warncke, K. Reiter, J. Williams, A. Ziegler, A. Bingley, P. Bonifacio, E. (2006): Type 1 diabetes risk assessment: improvement by follow-up measurements in young islet autoantibody-positive relatives. Diabetologia. 49:2969–2976.

Affana, W. (2016): Association between B12 and iron levels among patients suffering from *Helicobacter pylori* infection in Gaza strip. Master Thesis, AL-Azhar University of Gaza, Gaza strip, Palestine.

Al-Bayati, S.M. and Yas, A. (2010): Helicobacter Pylori Infection in Diabetic Patients. Journal of Medical Microbiology. 9(1):31-4.

ALhonen, S. Korhonen, S. Tapanainen, P. Knip, M. Veijola, R. (2011): Extended family history of diabetes and autoimmune diseases in children with and without type 1 diabetes. Diabetes Care. 34:115–117.

Aljabri, K.S. A. Bokhari, S.A. Alqurashi, K. (2013): The Prevalence of Autoantibodies in Saudis Patients with Type 1 Diabetes Mellitus. Journal of Endocrine and Metabolic Diseases. 3: 132-136.

Al-Muhammadi, O. Alwash, S.M. (2011): A Study of Some Haemato-Physiological Changes in Patients with Diabetic Nephropathy. Medical Journal of Babylon. 8(3): 461-475.

AL-Suhaimi, E. A. AL-Kulaifi1, F. M. Ravinayagam, V. Al-Qahtani, M.H. (2012): Serum Adipocytokines, Metabolic and Immunological Correlations in Type 1 Diabetes mellitus (T1DM) Children. The Open Endocrinology Journal. 6: 110-116.

American Diabetes Association, ADA. (2012): Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 35(1): 64-71.

American Diabetes Association, ADA. (2013): Standards of Medical Care in Diabetes—2013. Diabetes Care. 36(1): 11-66.

American Diabetes Association, ADA. (2014): Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 37(1): S81–S90.

Arslan, D. Kendirci, M. Kurtoglu, S. Kula, M.(2000): Helicobacter pylori infection in children with insulin dependent diabetes mellitus. Journal of Pediatric Endocrinol Metabolism. 13(5):553-6.

Assal, A.H. Gad, M.A. El Badawy, R.M. Emar, N.M. andSoliman, M.S. (2013): The Association between *Helicobacter pylori* Infection and Insulin Resistance. The International Medical Journal Malaysia. 12 (1): 49-52.

Atiba, A.S. Oparinde, D.P. Babatunde, O.A. Niran-Atiba, T.A. Jimoh, A.K and Adepeju, A.A. (2013). Liver enzymes and lipid profile among type 2 diabetic patients in Osogbo, Nigeria. Greener Journal of Medical Sciences 3(5), 174-178.

Bánhidy, F.; Acs, N.; Puhó, EH.; et al. (2011): Chronic hypertension with related drug treatment of pregnant women and congenital abnormalities in their offspring: a population-based study. Hypertension Research: Official Journal of the Japanese Society of Hypertension 34:257–63.

Bener, A. Micallef, R. Afifi, M. Derbala, M. Al-Mulla, H.M. and Usmani, M.A. (2007): Association between type 2 diabetes mellitus and *Helicobacter pylori* infection. Turkish Journal of Gastroenterology. 18(4): 225-229.

Bishop, F.K. Wadwa, R.P. Snell-Bergeon, J. Nguyen, N. and Maahs, D.M. (2014): Changes in diet and physical activity in adolescents with and without type 1 diabetes over time. International Journal of Pediatric Endocrinology 2014(17):1-7.

Block, C.E. Leeuw, I.H. and Van Gaal L.F. (2008): Autoimmune gastritis in type 1 diabetes: a clinically oriented review. Journal of Clinical Endocrinology & Metabolism. 93(2):363-371.

Bluestone, JA. Herold, K. Eisenbarth, G. (2010): Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature. 464(7293): 1293–1300.

Bonifacio, E. Hummel, M. Walter, M. Schmid, S. Ziegler, A. (2004): IDDM1 and Multiple Family History of Type 1 Diabetes Combine to Identify Neonates at High Risk for Type 1 Diabetes. Diabetes care. 27(11): 2695- 2700

Brown, L.M. (2000): *Helicobacter pylori*: epidemiology and routes of transmission. Epidemiologic Reviews. 22(2): 283-297.

Bruno Vergès (2011). Lipid Disorders in Type 1 Diabetes, Type1Diabetes-Complications, Pathogenesis, and Alternative Treatments, Chih-Pin L. (Ed.), ISBN: 978-953-307-756-7, InTech, Available from: *http://www.intechopen.com/books/type-1-diabetes-complications pathogenesis-and-alternative treatments/lipid disorders-in-type-1-diabetes.*

Bucolo, G. and David, H. (1973): Quantitative determination of serum triglycerides by the use of enzymes. Clinical Chemistry Journal. 19(5):476-482.

Candelli, M. Rigante, D. Schiavino, S. Gabrielli, M. Crea, F. Mingeull Del Lungo, L. Pignataro, G. Sacco, E. Monaco, S. Gentilonia Silveri, N. Gasbarrini, A. (2012) High reinfection rate of Helicobacter pylori in young type 1 diabetic patients: a three-year follow-up study. European Review for Medical and Pharmacological Sciences, 16, 1468–1472.

Carrera Boada, C. A and Martínez-Moreno, J. M. (2013): Pathophysiology of diabetes mellitus type 2: beyond the duo "insulin resistance-secretion deficit" . Nutricion Hospitalaria. 28(2):78-87.

Charles, A. Janeway, J.R. Travers, P. Walport, M. and Shlomchik, M.J. (2005): Immunobiology, The Immune System in Health. and Disease. 6th edition.

Chen, L.K. Lin, M.H. Chen, Z.J. Hwang, S.J. and Chiou, S.T. (2006): Association of Insulin Resistance and Hematologic Parameters: Study of a Middle-aged and Elderly Chinese Population in Taiwan. Journal of the Chinese Medical Association. 69(6): 248-253.

Chen, M.Y. He, C.Y. Meng, X. and Yuan, Y. (2013): Association of *Helicobacter pylori* babA2 with peptic ulcer disease and gastric cancer. World Journal of Gastroenterology.19(26): 4242-4251.

Chen, Y. and Blaser, M.J. (2012): Association between gastric *Helicobacter pylori* colonization and glycated hemoglobin levels. The Journal of Infectious Diseases. 205(8):1195-1202.

Cho, N.H. Jang, H.C. Choi, S.H. Kim, H.R. Lee, H.K. Chan, J.C. andLim, S. (2007). Abnormal liver function test predicts type 2 diabetes: a community-based prospective study. Diabetes Care 30(10), 2566-2568.

Choudhary, M. Jinger, M.K. Gahlot, Y.G. Saxena, R. (2014): Comparative study of liver function test in type 1 and type 2 diabetes mellitus. Indian Journal of Scientific Research 5(2):143-147.

Chu, Y. T., Wang, Y. H., Wu, J. J. & Lei, H. Y. (2010): Invasion and multiplication of *Helicobacter pylori* in gastric epithelial cells and implications for antibiotic resistance. Infection and Immunity Journal 78, 4157–4165.

Cobuz, M. Cobuz, C. Chronic complications of type 1 diabetes mellitus in children. Romanian Journal of Diabetes Nutrition and Metabolic. 19(3):301-309.

Cohen, D. and Muhsen, K. (2012): Association Between *Helicobacter pylori* Colonization and Glycated Hemoglobin Levels: Is This Another Reason to Eradicate *H. pylori* in Adulthood? Journal of Infectious Diseases Advance. 208(12):1-5.

Conget, I. (2002): Diagnosis, classification and pathogenesis of diabetes mellitus. Revista Española de Cardiología. 55(5): 528–535.

Dashty M (2014) A Quick Look at Biochemistry: Lipid Metabolism. Journal of Diabetes & Metabolism. 5(1): 2: 324. doi:10.4172/2155-6156.1000324.

DeFronzo, R. A., Davidson, J. A., & del Prato, S. (2012). The role of the kidneys in glucose homeostasis: A new path towards normalizing glycaemia. Diabetes, Obesity & Metabolism. 14(1): 5–14.

Delport, W. and van der Merwe, S.W. (2007): The transmission of *Helicobacter pylori*: the effects of analysis method and study population on inference. Best Practice & Research Clinical Gastroenterology. 21(2): 215–236.

De Luis, DA. De la Calle, H. Roy, G. de Argila, CM. Valdezate, S. Canton, R. Boixeda, D.(1997): Helicobacter pylori infection and insulin-dependent diabetes mellitus. Diabetes Research and Clinical Practice - Journal. 39(2):143-6.

d'Emden, M.C. Shaw, J.E. Colman, P.G. Colagiuri, S. Twigg, S.M. Jones, G. Goodall, I. Schneider, H.G. Cheung, N.W. (2012): The role of HbA1c in the diagnosis of diabetes mellitus in Australia. Medical Journal of Australia. 197(4): 220-221.

Demir, M. Gokturk, H.S. Ozturk, N.A. Kulaksizoglu, M. Serin, E. Yilmaz, U. (2008): *Helicobacter pylori* prevalence in diabetes mellitus patients with dyspeptic symptoms and its relationship to glycemic control and late complications. Digestive Diseases and Sciences. 53(10): 2646-2649.

El-Eshmawy, M.M. El-Hawary, A.K. Abdel Gawad, S.S. El-Baiomy, A.A. (2011): Helicobacter pylori infection might be responsible for the interconnection between

type 1 diabetes and autoimmune thyroiditis. Diabetology & Metabolic Syndrome. 3(28): 1-7.

Elhamalawi, I (2015): Assessment of serum Vitamin D in type 1 diabetic patients from gaza strip. Master Thesis, Islamic University of Gaza, Gaza strip, Palestine.

Enroth, H. andWreiber. K. (1999): In vitro aging of *Helicobacter pylori*: changes in morphology, intracellular composition and surface properties. Helicobacter. 4(1): 7-16.

Escobar, M.L. and Kawakami, E. (2004): Evidence of motherchild transmission of *Helicobacter pylori* infection. Arquivos de Gastroenterologia. 41(4): 239 244.

Fawcett, J.K. and Scott, J.E. (1960): A rapid and precise method for the determination of urea. Journal of Clinical Pathology. 13(2):156-159.

Fayed, SB. Abd El Dayem, SM. Khalil,E. Abd El Kader, M. Abd El Halim, E. (2014): *Helicobacter Pylori* Infection in Children with Type 1 Diabetes Mellitus. Macedonian Journal of Medical Sciences. 7(1):114-118.

Fox, J. (2002): The non-*H. pylori* helicobacters: their expanding role in gastrointestinal and systemic diseases. Gut. 50(2): 273-283.

Furth, A.J. (1997): Glycated proteins in diabetes. British Journal of Biomedical Sciences. 54(3): 192-200.

Galler, A. Lindau, M. Ernert, A. Thalemann, R. Raile, K. (2011): Associations between media consumption habits, physical activity, socioeconomic status, and glycemic control in children, adolescents, and young adults with type 1 diabetes. Diabetes Care.34 (11): 2356-2359.

Goldenberg, R. Punthakee, Z. (2013): Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome. Canadian Journal of Diabetes 37: S8-S11.

Goldstein, D.E. Little, R.R. Lorenz, RA. Malone, J.I. Nathan, D. Peterson, C.M. (2004): Tests of glycemia in diabetes. Diabetes Care. 27(1): 1761–1763.

Goodwin, C.S &Worsley, B.W. (1993): Microbiology of *Helicobacter pylori*. Gastroenterology Clinics of North America. 22(1): 5–19.

Goodwin, C.S. Armstrong, J.A. Chilvers, T. (1989): Transfer of *Campylobacter pylori* and *Campylobacter mustelae* to *Helicobacter* gen. nov. as *Helicobacter pylori* comb. nov. and *Helicobacter mustelae* comb. novRespectively. International journal of systematic bacteriology. 39(4): 397–405.

Grove, T.H. (1979): Effect of reagent pH on determination of high-density lipoprotein cholesterol by precipitation with sodium phosphotungstatemagnesium. Clinical Chemistry. 25(4):560 564.

Harjutsalo, V. Reunanen, A. and Tuomilehto, J. (2006): Differential transmission of type 1 diabetes from diabetic fathers and mothers to their offspring. Diabetes. 55(5): 1517-1524.

Hassan BAR (2013) Overview on Diabetes Mellitus (Type 2). Journal of Chromatography & Separation Techniques. 4(3): e114.

Iida, M. Ikeda, F. Ninomiya, T. Yonemoto, K. Doi, Y. Hata, J. Matsumoto, T. Iida, M. and Kiyohara, Y. (2012): White Blood Cell Count and Risk of Gastric Cancer Incidence in a General Japanese Population. American Journal of Epidemiology Advance. 175(6): 504-510.

International Diabetes Federation, IDF. (2009): Diabetes Atlas. 4th edn. Brusesls: International Diabetes Federation.

International Diabetes Federation, IDF. (2011): Diabetes Atlas. 5th edn. Brusesls: International Diabetes Federation.

Jafarzadeh, A. Ahmedi-Kahanali, J. Bahrami, M. and Taghipour, Z. (2007): Seroprevalence of anti-*Helicobacter pylori* and anti-CagA antibodies among healthy children according to age, sex, ABO blood groups and Rh status in south-east of Iran. The Turkish Journal of Gastroenterology. 18(3): 165-171.

Jafarzadeh, A. Akbarpoor, V. Nabizadeh, M. Nemati, M. and Rezayati, M.T. (2013): Total Leukocyte Counts and Neutrophil-Lymphocyte Count Ratios Among Helicobacter pylori-Infected Patients with Peptic Ulcers. Southeast Asian journal of tropical medicine and public health44(1): 82-88.

Jeon, C.Y. Haan, M.N. Cheng, C. Clayton. E.R. Mayeda, E.R. Miller, J.W. and Aiello, A.E. (2012): *Helicobacter pylori* Infection Is Associated With an Increased Rate of Diabetes. Diabetes Care. 35(3): 520-525.

Khalifa, M.M. Sharaf, R.R. and Aziz, R.K. (2010): *Helicobacter pylori*: a poor man's gut pathogen? Gut Pathogens. 2: 2.

Kantárová, D. Buc, M.(2007): Genetic susceptibility to type 1 diabetes mellitus in humans. Physiol Research. 56(3):255-66.

Kapellen, T.M. Gausche, R. Dost, A. Wiegand, S. Flechtner-Mors, M. Keller, E. Kiess, W. Holl, R.W. (2014): Children and adolescents with type 1 diabetes in Germany are more overweight than healthy controls: results comparing DPV database and CrescNet database. Journal of pediatric endocrinology & metabolism. 27(3-4):209-14.

Kate, V. Maroju, N.K. and Ananthakrishnan, N. (2013): *Helicobacter pylori* Infection and Upper Gastrointestinal Disorders. Gastroenterology Research and Practice. 1-3.

Kayar,Y. Pamukçu, Ö. EroLlu, H. Erol, K. Ilhan, A. Kocaman1, O. (2015): Relationship between Helicobacter pylori Infections in Diabetic Patients and Inflammations, Metabolic Syndrome, and Complications. International Journal of Chronic Diseases.1-6.

Kothari, R. Bokariya, P. (2012): A Comparative Study of Haematological Parameters in Type I d i a b e t e s mellitus Patients & healthy Young Adolescents. International Journal of Biological and Medical Research. 3(4): 2429-2432

Kuczmarski, R.J. Ogden, C.L. Grummer-Strawn, L.M. Flegal, K.M. Guo, S.S. Wei, R. Mei, Z. Curtin, L.R. Roche, A.F. Johnson, C.L.(2000): CDC growth charts for the United States: Methods and development. National Center for Health Statistics, Vital Health Stat. Advanced Data. 8(314): 1–28.

Kumaran, C. Mohana, R. L. (2014): Relation between lipid profile changes and *Helicobacter pylori* Infection. Indo American Journal of Pharm Research. 4(05):2435-2437 .

Kusters, J. Vanvilet, A. and Kuipers, E. (2006): Pathogenesis of *Helicobacter pylori* infection. Clinical Microbiology Reviews. 19(3): 449-490.

Leeds, J.S. Forman, E.M. Morley, S. Scott, A.R. Tesfaye, S. Sanders, D.S.(2009): Abnormal liver function tests in patients with Type 1 diabetes mellitus: prevalence, clinical correlations and underlying pathologies. Diabetic Medicine. 26(12):1235-41.

Lehours, P. and Yilmaz, O. (2007): Epidemiology of *Helicobacter pylori* Infection. Journal compilation. Blackwell. Helicobacter. 12(1): 1-3.

Lozano, R. Naghavi, M. Foreman, K. et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380(9859):2095-2128.

Mehers, K.L. and Gillespie, K.M. (2008): The genetic basis for type 1 diabetes. British Medical Bulletin. 88: 115–129.

Maori, L. Ezekiel, D. Bilal, J. Alibe, W. Haruna, L. (2014): Prevalence of Diabetes Mellitus in Patients Attending Zambuk General Hospital. Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 9(2): 08-11.

Manco, M. Putignani, L. and Bottazzo, G.F. (2010): Gut microbiota, lipopolysaccharides, and innate immunity in the pathogenesis of obesity and cardiovascular risk. Endocrine Reviews Journal. 31(6): 817–844.

Mansour, Ramzi. (2014): Assessment of *Helicobacter pylori* Infection as a Risk Factor for Coronary Artery Disease in Gaza Strip. Master thesis Islamic University-Gaza.

Marshall, B. and Warren, J. (1984): Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet. 1(8390): 1311-1315.

Masram, S.W. Bimanpalli, M.V. Ghangle, S. (2012): Study of Lipid Profile and Glycated Hemoglobin in Diabetes Mellitus. Indian Medical Gazette. 145(7):257-265.

Mehmood, M. Shahab-uddin, M.A. Ahmed, A. Usmanghani, K. Hannan, A. Mohiuddin, E. and Asif,M. (2010): *Helicobacter pylori*: an introduction. International Journal of Applied Biology and Pharmaceutical Technology. 1(3): 1337-1351.

Meiattini, F. Prencipe, L. Bardelli, F. Giannini, G. and Tarli, P. (1978): The 4-hydroxybenzoate/4-aminophenazone chromogenic system used in the enzymic determination of serum cholesterol. Clinical Chemistry. 24(12): 2161-2165.

Meissner, T. Wolf, J. Kersting, M. Fröhlich-Reiterer, E. Flechtner-Mors, M. Salgin, B. Stahl-Pehe, A. Holl, R.W. Carbohydrate intake in relation to BMI, HbA1c and lipid profile in children and adolescents with type 1 diabetes. Clinical Nutrition - Journal. 33(1):75-8.

Ministry of Health, MOH. (2005): Health status in Palestine, Annual Report 2004, State of Palestine, Health Management Information Center.

Ministry of Health, MOH. (2012): Health Status in Palestine, Annual Report2011, State of Palestine, Health Management Information Center.

Ministry of Health, MOH. (2014): Chronic disease, Annual Report2014, State of Palestine, Palestinian Health Information Center.

Ministry of Health, MOH. (2014): Mortality rate in Palestine, Annual Report2014, State of Palestine, Palestinian Health Information Center.

Mishra, S. Singh, V. Rao, G.R. Jain, A.K. Dixit, V.K. Gulati, A.K. and Nath, G. (2008): Detection of *Helicobacter pylori* in stool specimens: comparative evaluation of nested PCR and antigen detection. Journal of Infection in Developing Countries. 2(3): 206- 210.

Mohamady,O. Ramadan,N. Arnaout, H.(2013): The prevalence of helicobacter pylori infection in diabetic patients and its relation to the presence of gastrointestinal tract complications. International Journal of Academic Research. 5(4), 201-209.

Momtaz, H. Souod, N.Dabiri, H. and Sarshar. M. (2012): Study of *Helicobacter pylori* genotype status in saliva, dental plaques, stool and gastric biopsy samples. World Journal Gastroenterol. 18(17): 2105–2111.

Morgner, A. Bayrdorffer, E. Neubauer, A. and Stolte, M. (2000): Gastric mucosa-associated lymphoid tissue lymphoma and *helicobacter pylori*. Malignant tumors of the stomach. Gastroenterology Clinics of North America. 29(3): 593- 607.

National Committee for Clinical Laboratory Standards, NCCLS. (2001): Protection of Laboratory Workers from Occupationally Acquired infection: Approved Guideline . 2nd ed. Document M29-A2. Wayne, PA: NCCLS.

Newman, D.J. and Price, C.P. (1999): Renal function and nitrogen metabolites. In: Burtis C.A, Ashwood E.R, (eds) Tietz Text book of clinical chemistry. 3rdedition, Philadelphia: W.B Standers Company. 1204-1207.

Nguyen, T.L. Uchida, T. Tsukamoto, Y. Trinh, D.T. Ta, L. Mai, B.H. Le, S.H. Thai, K.D. Ho, D.D. Hoang, H.H. Matsuhisa, T. Okimoto, T. Kodama, M. Murakami, K. Fujioka, T. Yamaoka, Y. and Moriyama, M. (2010): *Helicobacter pylori* infection and

gastroduodenal diseases in Vietnam: a cross-sectional, hospital-based study. BMC Gastroenterol. 10(114): 1-7.

Nurgalieva, Z.Z. Malaty, H.M. Graham, D.Y. Almuchambetova, R. Machmudovaet, A. andKapsultanova, D. (2002): *Helicobacter pylori* infection in Kazakistan: effect of water sourceand household hygiene. The American Journal of Tropical Medicine and Hygiene. 67(2): 201-206.

Oldenburger, B. Diepersloot, R.J. and Hoekstra, J.B. (1996): High seroprevalence of *Helicobacter pylori* in diabetes mellitus patients. Digestive Diseases and Sciences. 41(3): 458-461.

Ojetti, V1. Pitocco, D. Ghirlanda, G. Gasbarrini, G. Gasbarrini, A. (2002): Role of Helicobacter pylori infection in insulin-dependent diabetes mellitus. Minerva Medica. 92(3):137-44.

Oona, M. Utt ,M. Nilsson, I. Uibo, O. Vorobjova, T. (2004): *Helicobacter pylori* infection in children in Estonia: decreasing seroprevalence during the 11-year period of profoundsocioeconomicchanges. Helicobacter. 9(3): 233–241.

Organization for Economic Co-operation and Development, (OECD, 2013): Diabetes prevalence and incidence. In health at a Glance 2013: OECD indicators, OEDC publishing.

Oyedemi, S.O. Yakubu, M.T. Afolayan, A.J. (2011): Antidiabetic activities of aqueous leaves ex- tract of Leonotis leonurus in streptozotocin induced diabetic rats. Asian Pacific Journal of Tropical Biomedicine. 1(5): 353–358.

Ozer, J. Ratner, M. Shaw, M. Bailey, W. and Schomaker, S. (2008). The current state of serum biomarkers of hepatotoxicity. Toxicology 245(3), 194-205.

Ozougwu, J. C. Obimba, K. C. Belonwu, C. D. and Unakalamba, C. B. (2013): The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. Journal of Physiology and Pathophysiology. 4(4) 46-57.

Palestinian clinical laboratory tests guide, PCLTG. (2005): Ministry of Health Palestine, MOH. first edition.

Palestinian Ministry of Health (2011): Health Report, Palestine Mid-Year 2011, Palestinian Health Information Center,Septemper , 2011.

Parkkola, A. Härkönen,T. Ryhänen, S. Ilonen, J. Knip, M. (2013): Extended Family History of Type 1 Diabetes and Phenotype and Genotype of Newly Diagnosed Children. Diabetes care. 36(2): 348-354.

Patterson, C. C1. Dahlquist, G. G. Gyürüs, E . Green, A . Soltész, G. (2009): Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. Lancet. 373(9680):2027-2033.

Peek, R.M. (2004): *Helicobacter pylori* and Gastroesophageal Reflux Disease. Current Treatment Options in Gastroenterology. 7(1): 59-70.

Phillips, P.J. Leow, S. (2014): HbA1c, blood glucose monitoring and insulin therapy. Australian family physician. 43(9): 611-615.

Raju SM, Raju B (2010). Illustrated medical biochemistry. 2nd Edition. Jaypee Brothers Medical Publishers ltd, New Delhi, India. 645pp.

Rasmussen, L.T Labio, R.W. Gatti, L.L Silva, L.C. Queiroz, V.F. Smith, A. Payão, S.L. (2010): *Helicobacter pylori* detection in gastric biopsies, saliva and dental plaque of Brazilian dyspeptic patients. Memórias do Instituto Oswaldo Cruz. 105(3): 326-330.

Sachs, G. and Scott, R.D. (2012): *Helicobacter pylori*: Eradication or Preservation. Medical representatives. 4(7): 1-18.

Salardi, S. Cacciari, E. Menegatti, M. Landi, F. Mazzanti, L. Stella, FA. Pirazzoli, P. Vaira, D. (1999): Helicobacter pylori and type 1 diabetes mellitus in children. Journal of Pediatric Gastroenterology and Nutrition. 28(3):307-9.

Seniuk, K.W. Ozegowska, E.W. and Szczapa, J. (2009): Long-term effects of diabetes during pregnancy on the offspring. Pediatric Diabetes. 10(7): 432–440.

Shiota, S. Murakawi, K. and Yamaoka, Y. (2013): *Helicobacter pylori* infection in Japan. Expert Review of Gastroenterology & Hepatology. 7(1): 35-40.

Shamsi, B. Hashemipour, M. Saadat, S. Emami, S. Abdyazdan, Z. Hasanzadeh, A. Khaibi, K. Haghighi , S. Hovsepian, S. (2004): Prevalence of *Helicobacter pylori* infection in type 1 diabetic children refereeing to isfahan endocrine & metabolism research center. Iranian journal of diabetes and lipid disorders. 3(1): 35-40.

Silva, D.G. Stevens, R.H. Macedo, J.M. Albano R.M. Falabella, M.E. Veerman, E.C. and Tinoco, E.M. (2009): Detection of cytotoxin genotypes of *Helicobacter pylori* in stomach, saliva and dental plaque. Archives of Oral Biology. 54(7): 684-688.

Siva1, L. Mythili, S.V. Rani, J. Sai Kumar, P. (2012): Biochemical and Haematological Aberrations in Type I and Type II Diabetic Patients in South India-A Comparative Study. International Journal of Pharma and Bio Sciences. 3(2): 967-977.

Sousa, L. Vásquez, L. Velasco, J. Parlapiano, D. (2006): Isolation of *Helicobacter pylori* in gastric mucosa, dental plaque and saliva in a population from the Venezuelan Andes. Clinical Investigation. 47(2): 109-116.

Stasi, R. andProvan, D. (2008): *Helicobacter pylori* and Chronic ITP. Hematology Hematology-American Society of Hematology Education Program. 1: 206-211.

Stenström, B. Mendis, A. and Marshall, B. (2008): *Helicobacter pylori* the latest in diagnosis and treatment. Australian Family Physician. 37(8): 608–612.

Suerbaum, S.and Michetti, P. (2002): *Helicobacter pylori* infection. The New England Journal of Medicine. 347(15): 1175-1186.

AL-Suhaimi, E.A. AL-Kulaifi, F.M. Ravinayagam, V andAl-Qahtani, M.H. (2012): Serum Adipocytokines, Metabolic and Immunological Correlations in Type 1 Diabetes mellitus (T1DM) Children. The Open Endocrinology Journal.(6); 110-116.

Sumathi, K. and Kalaiselvi, V. (2014): Estimation of Microalbuminuria in Hearing Loss in Diabetes Mellitus – A Review. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 5(5):936-940.

Szadkowska, A. Madej, A. Ziółkowska, K. Szymańska, M. Jeziorny, K. Mianowska, B. Pietrzak, I. (2015): Gender and Age – Dependent effect of type 1 diabetes on obesity and altered body composition in young adults. Annals of Agricultural and Environmental Medicine. 22(1): 124–128.

Taher, M. Mashayekhi, M. Hashemi, M. and Bahrani, V. (2012): *Helicobacter pylori* in Diabetic and Non-Diabetic Patients with Dyspepsia. Acta Medica Iranica. 50(5): 315-318.

Takuma, Y. (2011). *Helicobacter pylori* infection and liver diseases. Gan to Kagaku Ryoho - Japanese Journal of Cancer and Chemotherapy 38(3), 362-364.

Tanriverd, Ö. (2011): Association of Helicobacter pylori infection with microalbuminuria in type 2 diabetic patients. Turkish Journal of Gastroenterology. 22(6): 569-574.

Thomas, L. (1998): Clinical Laboratory Diagnostics. 1st ed. Frankfurt: THBooks Verlagsgesellschaft.

Thomas, S. Rampersad, M. (2004): Anaemia in diabetes. Acta Diabetol 41 Suppl 1: 13-17.

Tiwari, S.K. Khan, A.A. Ahmed, KS. Ahmed, I. Kauser, F. and Hussain, M.A. (2005): Rapid diagnosis of *Helicobacter pylori infection* in dyspeptic patient using salivary secretion. A non-invasive approach. Singapore Medical Journal. 46(5): 224-228.

Toporowska-Kowalska, E. Wasowska-Królikowska, K. Szadkowska, A. Bodalski, J. (2007): Helicobacter pylori infection and its metabolic consequences in children and adolescents with type 1 diabetes mellitus. Medycyna wieku rozwojowego Journal. 11(2):103-8.

Travis, J.C. (1980): Clinical Radioimmunoassay. State of the Art. Anaheim. Scientific Newsletters, Inc. Radioassay-Ligand Assay, Publishers 89-92.

Trinder, P. (1969): Glucose GOD-PAP enzymatic and colorimetric method. Annals of Clinical Biochemistry. 6: 24.

Trivelli, L.A. Ranney, H.M. and Lai, H.T. (1971): Hemoglobin components in patients with diabetes mellitus. The New England Journal of Medicine. 284(7):353-357.

Türkay, C. Erbayrak, M. Bavbek, N. Yenıdünya, S. Eraslan, E. Kasapoğlu, B. (2011): *Helicobacter pylori* and histopathological findings in patients with dyspepsia. The Turkish Journal of Gastroenterology. 22(2): 122-127.

Uko, E.K. Erhabor, O. Isaac, I.Z. Abdulrahaman, Y. Adias, T.C. Sani, Y. Shehu, R.S. Liman, H.M. Dalltu, M.K. Mainasara, A.S. (2013): Some Haematological Parameters in Patients with Type-1 Diabetes in Sokoto, North Western Nigeria. J Blood Lymph 3: 110.

Vale, F.F. and Vítor, J.M. (2010): Transmission pathway of *Helicobacter pylori*: does food play a role in rural and urban areas? International Journal of Food Microbiology. 138(1-2): 1-12.

Van Belle, TL. Coppieters, KT. von Herrath, MG. (2011): Type 1 Diabetes: Etiology, Immunology, and Therapeutic Strategies. *Physiological Reviews* 91: 79–118.

Van Zwet, A.A. Thijs, J.C. Kooistra-Smid, A.M.(1994): Use of PCR with fecesfor detection of *Helicobacter pylori* infections in patients. Journal of Clinical Microbiology, 32(5): 1346–1348.

Wang, F. Liu, J. LV, Z. (2013): Association of Helicobacter pylori infection with diabetes mellitus and diabetic nephropathy: A meta-analysis of 39 studies involving more than 20,000 participants. Scandinavian Journal of Infectious Diseases. 45: 930–938.

Warren, J.R and Marshall, B. (1983): Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet . 321(8336): 1273-1275.

Wellen, K.E. and Hotamisligil, G.S. (2005): Inflammation, stress, and diabetes. Journal of Clinical Investigation. 115(5): 1111–1119.

West, J. Brousil, J. Gazis, A. Jackson, L. Mansell, P. Bennett, A. Aithal, G.P.(2006): Elevated serum alanine transaminase in patients with type 1 or type 2 diabetes mellitus. Quarterly Journal of medicin. 99:871–876.

Witkowska, M. and Smolewski, P. (2013): *Helicobacter pylori I*nfection, Chronic Inflammation, and Genomic Transformations in Gastric MALT Lymphoma. Mediators of Inflammation Journal. 2013: 523170.

Wolpert, H.A. Atakov-Castillo, A. Smith, S. A. Steil, G. M. (2013): Dietary fat acutely increases glucose concentrations and insulin requirements in patients with type 1 diabetes . Diabetes Care. 36:810–816.

World Health Organization, (2006): Definition and Diagnosis Of Diabetes Mellitus And Intermediate Hyperglycemia. Report of a WHO/IDF Consultation. Geneva, World Health Organization.

World Health Organization, WHO. (2011): Report of a World Health Organization Consultation Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of DiabetesMellitus. Diabetes Research and Clinical Practice 93: 299- 309.

Yassin, M. Altibi, H. El Shanti, A. (2011): Clinical and biochemical features of type 2 diabetic patients in Gaza Governorate, Gaza Strip. West African Journal Medicine. 30(1): 51-56.

Yassin, M.M. H. Lubbad, AM. Saadallah, N.M. AbuTaha, A.J. (2014): Biochemical and hematological parameters in relation to Helicobacter pylori infection among type 2 diabetic patients in Gaza Strip. Journal of Advanced Medical Research.4 (1):10-20.

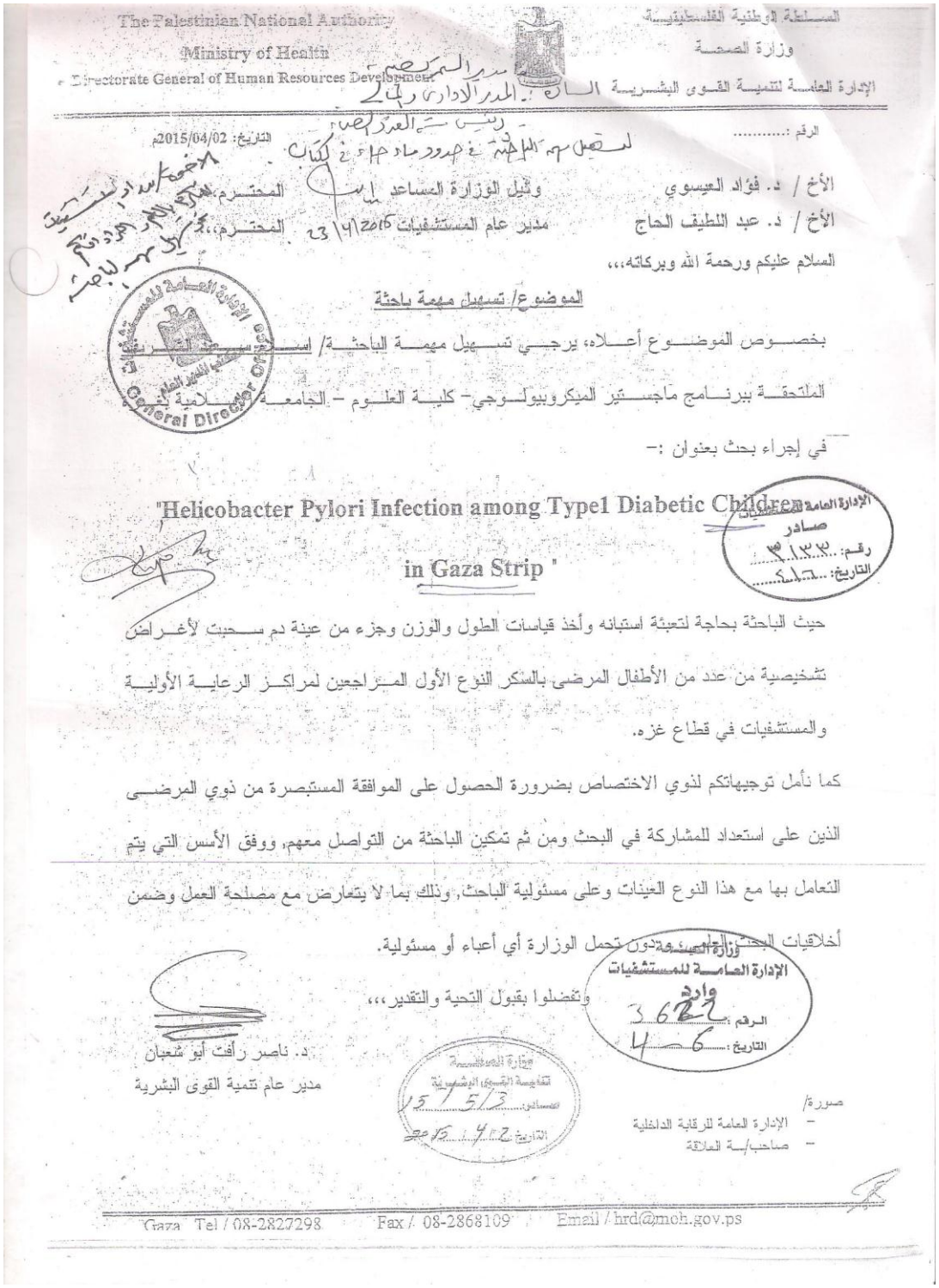
Zekrya, O.A and Abd Elwahid, H.A.(2013): The association between Helicobacter pylori infection, type 1 diabetes mellitus, and autoimmune thyroiditis. Journal of the Egyptian Public Health Association. 88:143–147.

Zhao Y, Jiang Z, Zhao T, et al. (2012): Reversal of type 1 diabetes via islet beta cell regeneration following immune modulation by cord blood-derived multipotent stem cells. BMC Medicine. 10:3.

Zhou, S. Xu, L. Wang, B. Fan, X. Wu, J. and Wang, C. (2012): Modified sequential therapy regimen versus conventional triple therapy for *Helicobacter pylori* eradication in duodenal ulcer patients in China. A multicenter clinical comparative. Gastroenterology Research and Practice. 2012: 405-425.

Zhou, X. Zhang, C. Wu, J. Zhang, G. (2013): Association between *Helicobacter pylori* infection and diabetes mellitus: a meta-analysis of observational studies. Diabetes Research and Clinical Practice. 99(2): 200-208.

Annex 1: Ministry of Health permission letter



السلطة الوطنية الفلسطينية
وزارة الصحة
الإدارة العامة لتنمية القوى البشرية

The Palestinian National Authority
Ministry of Health
Directorate General of Human Resources Development

الرقم: .....
التاريخ: 2015/04/02م

الأخ / د. فؤاد العيسوي — وكيل الوزارة المساعد
الأخ / د. عبد اللطيف الحاج — مدير عام المستشفيات

السلام عليكم ورحمة الله وبركاته،،،

الموضوع/ تسهيل مهمة باحثة

بخصوص الموضوع أعلاه، يرجى تسهيل مهمة الباحثة/ [illegible] الملتحقة ببرنامج ماجستير الميكروبيولوجي- كلية العلوم - الجامعة الإسلامية في [illegible] في إجراء بحث بعنوان :-

"Helicobacter Pylori Infection among Type1 Diabetic Children in Gaza Strip"

حيث الباحثة بحاجة لتعبئة استبانه وأخذ قياسات الطول والوزن وجزء من عينة دم سحبت لأغراض تشخيصية من عدد من الأطفال المرضى بالسكر النوع الأول المراجعين لمراكز الرعاية الأولية والمستشفيات في قطاع غزه.

كما نأمل توجيهاتكم لذوي الاختصاص بضرورة الحصول على الموافقة المستبصرة من ذوي المرضى الذين على استعداد للمشاركة في البحث ومن ثم تمكين الباحثة من التواصل معهم, ووفق الأسس التي يتم التعامل بها مع هذا النوع العينات وعلى مسئولية الباحث, وذلك بما لا يتعارض مع مصلحة العمل وضمن أخلاقيات البحث العلمي، دون تحمل الوزارة أي أعباء أو مسئولية.

وتفضلوا بقبول التحية والتقدير،،،

د. ناصر رأفت أبو شعبان
مدير عام تنمية القوى البشرية

صورة/
- الإدارة العامة للرقابة الداخلية
- صاحب/ـة العلاقة

Gaza Tel / 08-2827298 Fax / 08-2868109 Email / hrd@moh.gov.ps

Annex 2: Helsinki committee an approval letter

المجلس الفلسطيني للبحث الصحي
Palestinian Health Research Council

تعزيز النظام الصحي الفلسطيني من خلال مأسسة استخدام المعلومات البحثية في صنع القرار

"Developing the Palestinian health system through institutionalizing the use of information in decision making"

Helsinki Committee
For Ethical Approval

Date: 04/04/2016 **Number:** PHRC/HC/84/16

Name: Eslam S. AL Sharif **الاسم:** إسلام الشريف

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

نفيدكم علماً بأن اللجنة قد ناقشت مقترح دراستكم حول:

Helicobacter pylori infection among type 1 diabetic children in Gaza strip

The committee has decided to approve the above mentioned research. Approval number PHRC/HC/84/16 in its meeting on 04/04/2016

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

Signature

Member **Member**

Chairman

4/4/2016

Genral Conditions:-

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

Specific Conditions:-

E-Mail:pal.phrc@gmail.com

Gaza - Palestine غزة - فلسطين

Annex3: Questionnaire

Helicobacter pylori infection among type 1 diabetic children in Gaza strip.

أخي المواطن الكريم/أرجو مساعدتنا في اتمام هذه الدراسه (بحث ماجستير علوم حياتيه/الجامعه الاسلاميه)والتي وذلك للحد من تختص بمرضى النوع الاول من السكر، حيث ان هدفنا الوقوف على مسبباته، وخاصه علاقته بالجرثومه الملويه البوابيه (Helicobacter pylori).مضاعفاته.

Sociodemographic data

Name ……..

Code number ……

Age in years …….

Gender a. Male b. Female

What is the average family income per month (shekel)?
a. Less than 1000 b. 1000-2000 c. More than 2000

Family history of diabetes

Yes No

Diet

Yes No

Compliance of medication

Yes No

Clinical data

Duration of diabetes?

Self-reported complications

Cardiovascular disease	Yes	No
Retinopathy	Yes	No
Neuropathy	Yes	No
Gastritis	Yes	No
Peptic ulcer	Yes	No

Anthrpometric measurement

Height (cm):……..Weight (kg):…….

Body Mass Index:……..

Agreement:

I agree to complete this questionnaire concerning my health statement.

أنا موافق على تعبئه هذا الاستبيان الذي يتعلق بصحتي.

التوقيع:.....

التاريخ:.....

شكرا لكم على حسن تعاونكم

الباحثه/اسلام سعيد الشريف