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Assessment of *Helicobacter pylori* Infection as a Risk Factor for Coronary Artery Disease in Gaza Strip

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DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of university of other institute, except where due acknowledgement has been made in the text.

Name **Signature** **Date**

Ramzi Ali Mansour ***January 2014-1435***

DEDICATION

I dedicate this work to:

My beloved parents who have always supporting me

My brothers and sisters

My wife and sons Ali, Sara and Omar, 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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Assessment of *Helicobacter pylori* Infection as a Risk Factor for Coronary Artery Disease in Gaza Strip

Abstract

Background: Coronary artery disease (CAD) is a common form of cardiovascular disease (CVD). It caused by atherosclerosis, which restricts blood flow to the heart, and when the blood flow completely cut off, the result is heart attack. Cardiovascular disease remains the leading cause of death in the world as well as in Palestine. *Helicobacter pylori* (*H. pylori)* infection believed to be associated with CAD.

Objective: Assessment of *H. pylori* infection as a risk factor for CAD in Gaza strip.

Material and methods: This case-control study comprised 62 CAD patients (Cases: 31 males and 31 females) and 62 healthy controls (31 males and 31 females). Questionnaire interview was applied. Blood samples were collected, processed and analyzed. Serum *H. pylori* IgG, cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatinine phosphokinase (CK) and creatinine phosphokinase MB (CK_{MB}) were determined. White blood cell (WBC), red blood cell (RBC), hemoglobin (Hb) and platelet (PLT) were also determined. Data were analyzed using SPSS version 18.0.

Results: Coronary artery disease was more prevalent among less educated and unemployed individuals, families with low income, individuals with family history of the disease as well as among smokers. The number of cases who had diabetes mellitus, hypertension and peptic ulcer was significantly higher than that of controls. The BMI was significantly higher in cases compared to controls (31.7±4.8 vs 27.6±4.4, % difference=13.8 and P=0.000). There were significant elevations in the activities of serum AST and LDH in cases compared to controls (36.3±24.5 and 540.8±310.7 U/L vs 18.6±6.0 and

321.3±66.1 U/L, % difference=64.5 and 50.9, respectively P=0.000). Similarly CK and CKMB activities were higher in cases (225.7±216.1 and 22.7±15.5 U/L vs 101.2±50.0 and 11.4±4.9 U/L, % difference=76.2 and 67.8, respectively P=0.000). The levels of cholesterol, triglycerides and LDL-C were found to be higher in cases (208.9±47.6, 218.0±110.1 and 131.6±41.9 mg/dl, respectively) compared to controls (174.8±34.1, 167.4±57.7 and 104.4±31.2 mg/dl, % differences of 17.8%, 26.3%, and 23.1 and P=0.000, P=0.001, P=0.000 respectively). On the other hand, the level of HDL-C was significantly lower in cases (33.7±9.8 vs 37.6±8.4 mg/dl, % difference=10.9, P=0.020). The WBC count was significantly higher in cases compared to controls (9.80±3.3 vs 7.8±1.6 $\times 10^9$/L, % difference 22.7, P=0.000), whereas RBC count, hemoglobin content and PLT count did not show significant differences between cases and controls (P>0.05). The prevalence of *H. pylori* among CAD patients 46 (74.2%) was significantly higher than controls 26 (41.9%) with P=0.000. When related to *H. pylori*, serum triglycerides was significantly increased in *H. pylori* positive cases more than in negative cases (235.8±112.8 vs 166.6±85.7mg/dl, P=0.029), whereas HDL-C level was significantly lower in positive cases (31.7±8.0 vs 39.5±12.3 mg/dl, P=0.005). The WBC count was significantly higher in positive compared to negative cases (10.5± 3.5 vs 7.9±2.1 P=0.007).

Conclusions: *H. pylori* infection was significantly higher in CAD patients compared to controls. *H. pylori* infection was associated with higher triglyceride levels and WBC count, and lower HDL-C levels, and. Therefore, monitoring of *H. pylori* infection as a possible risk factor of coronary artery disease is of clinical value.

Keywords: *Helicobacter pylori*, coronary artery disease, Risk factor, Gaza Strip.

تقييم الإصابة بالجرثومة الملوية البوابية كعامل اختطار لمرض الشريان التاجي في قطاع غزة

ملخص الدراسة

مقدمة: مرض الشريان التاجي هو أكثر اشكال امراض القلب، وهو يحدث نتيجة لتصلب الشرايين الذي يمنع تدفق الدم للقلب، وعندما يتوقف الدم تماما تقف عضلة القلب، وتعد امراض القلب المؤدي الاول للوفاة في العالم وقطاع غزة، كما يعتقد بأن الإصابة بالجرثومة الملوية البوابية مرتبطة بمرض الشريان التاجي.

الهدف: تهدف الدراسة إلى تقييم الإصابة بالجرثومة الملوية البوابية كعامل اختطار لمرض الشريان التاجي في قطاع غزة.

الطرق والأدوات: اشتملت هذه الدراسة المشهدة على 62 حالة مرضية من مرضى الشريان التاجي (31 ذكور، 31 إناث) و62 شخصا من الأصحاء كعينات ضابطة (31 ذكور، 31 إناث)، وقد تم عمل مقابلات تم فيها تعبئة الاستبيانات، كذلك جمعت عينات الدم ثم تم معالجتاها وتحليلها، حيث تم قياس كل من الجلوبيولين المناعي ج للجرثومة الملوية البوابية، الكولسترول، الدهون الثلاثية، كولسترول البروتين الشحمي خفيض الكثافة، كولسترول البروتين الشحمي مرتفع الكثافة، انزيمات القلب. كذلك تم قياس كل من خلايا الدم البيضاء، خلايا الدم الحمراء، والصفائح الدموية محتوى الهيموجلوبين.

استخدم البرنامج الإحصائي SPSS-18.0 لتحليل البيانات والنتائج.

النتائج: بينت النتائج بأن مرض الشريان التاجي يعد أكثر انتشارا بين الأشخاص الأقل تعليما والأشخاص غير الموظفين والعائلات الأقل دخلا والأشخاص ذوي التاريخ العائلي للمرض بالإضافة إلى المدخنين. كما بينت النتائج وجود ارتفاع في انتشار مرض السكر وضغط الدم المرتفع والقرحة الهضمية عند الحالات المرضية مقارنة بالأصحاء، وهذه النتيجة ذات دلالة إحصائية. كما بينت النتائج وجود ارتفاع في مؤشر كتلة الجسم عند الحالات المرضية مقارنة بالأصحاء، وهذه النتيجة ذات دلالة إحصائية. كما بينت النتائج وجود زيادة ذات دلالة إحصائية في نشاط كل من انزيم (AST) وانزيم (LDH) في الحالات المرضية مقارنة بالأصحاء. بشكل مشابه وجود زيادة ذات دلالة إحصائية في نشاط كل من انزيم (CK) و انزيم (CKMB) في الحالات المرضية مقارنة بالأصحاء، كما أشارت النتائج إلى أن كل من الكولسترول والدهون الثلاثية و كولسترول البروتين الشحمي خفيض الكثافة قد ازدادت في الحالات المرضية مقارنة بالأصحاء، وهذه الزيادة ذات دلالة إحصائية. من ناحة أخرى وجد انخفاض في كولسترول البروتين الشحمي مرتفع الكثافة وهذه الزيادة ذات دلالة إحصائية، كما وجد ارتفاع ذو دلالة إحصائية في أعداد كرات الدم البيضاء عند الحالات المرضية مقارنة بالأصحاء، بينما لم تظهر النتائج فروقات ذات دلالة إحصائية في أعداد كرات الدم الحمراء ومحتوى الهيموجلوبين وأعداد الصفائح الدموية بين الحالات المرضية والأصحاء.

وأظهرت النتائج زيادة في انتشار الجرثومة الملوية البوابية بين مرضى الشريان التاجي مقارنة بالأصحاء، كما أظهرت النتائج وجود زيادة في مستويات الدهون الثلاثية عند الحالات المرضية المصحوبة بالجرثومة الملوية البوابية أكثر من الحالات المرضية الغير مصحوبة بالجرثومة الملوية البوابية، وهذه النتيجة ذات دلالة إحصائية، بينما وجد انخفاض ذو دلالة إحصائية في مستوى كولسترول البروتين الشحمي مرتفع الكثافة عند الحالات المرضية المصحوبة بالجرثومة. كذلك وجد ارتفاع ذو دلالة إحصائية في عدد كرات الدم البيضاء عند الحالات المرضية المصحوبة بالجرثومة الملوية البوابية.

الاستنتاجات: أشارت النتائج بأن الإصابة بالجرثومة الملوية البوابية أعلى عند مرضى الشريان التاجي بالمقارنة مع الأصحاء، وهذه النتيجة ذات دلالة إحصائية، وأظهرت النتائج بأن الإصابة بالجرثومة الملوية البوابية مقترنة بكل من الدهون الثلاثية، كولسترول البروتين الشحمي مرتفع الكثافة وعدد كرات الدم البيضاء، لذلك فإن التحكم بالإصابة بالجرثومة الملوية البوابية كعامل اختطار لمرض الشريان التاجي له قيمة سريريه.

الكلمات المفتاحية: الجرثومة الملوية البوابية، مرضى الشريان التاجي، عامل خطورة، قطاع غزة.

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List of Abbreviated Terms

Abbreviation	Full name
ACS	Acute coronary syndrome
AMI	Acute myocardial infarction
APIC	European prospective of cancer
AST	Aspartate aminotransferase
BMI	Body mass index
CAC	Coronary artery calcification
CAD	Coronary artery disease
CagA	Cytotoxin Associated Gene A
CBC	Complete blood count
CHD	Coronary heart disease
CK	Creatine kinase
CK_{MB}	Creatine kinase muscle brain
CRP	C-reactive protein
CVD	Cardiovascular diseases
DM	Diabetes mellitus
ECG	Electrocardiogram
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme Linked Immunosorbent Assay
H. pylori	*Helicobacter pylori*
Hb	Hemoglobin
HDL-C	High density lipoprotein cholesterol
IGT	Impaired glucose tolerance
IHD	Ischemic heart disease
LDH	Lactate dehydrogenase
LDL-C	Low density lipoprotein cholesterol
MALT	Mucosa associated lymphoid tissue
MI	Myocardial infarction
MOH	Ministry of Health
MVD	Multiple vessel disease

OxLDL	Oxidized low density lipoprotein
PCR	Polymerase chain reaction
PLT	Platelets
RBC	Red blood cell
RFS	Framingham risk factor
SES	Socioeconomic status
SPSS	Statistical package for social sciences
SVD	Single vessel disease
TG	Triglycerides
TNF	Tumor necrosis factor- α
VLDL-C	Very low density lipoprotein cholesterol
WBC	White blood cell
WHO	World Health Organization

Chapter 1

Introduction

1.1 Overview

Cardiovascular diseases (CVD) or heart diseases are the class of diseases that involve the heart or blood vessels**.** The various diseases that fall under the umbrella of heart disease include coronary artery disease (CAD), heart rhythm problems (arrhythmias), heart infections and heart defects including congenital heart defects **(Gaziano, 2005; Centers for Disease Control and Prevention CDC, 2009 and American Heart Association, AHA, 2013).** Heart failure is a common clinical syndrome that represents the final stage of a range of different heart diseases **(Haldeman et al., 1999 and Anderson et al., 2007).**

Coronary artery disease is a common form of CVD and it is the major source of morbidity and mortality in the developing and developed countries **(Walter, 2008)**. It is caused by atherosclerosis which restricts blood flow to the heart and when the blood flow is completely cut off, the result is heart attack **(CDC, 2009 and Sakakura, 2013).** The major risk factors involved in the development of atherosclerosis include hyperlipidemia, hypertension, smoking, and diabetes mellitus **(Marković et al. 2011)**. The pathogenesis of atherosclerosis involves the processes of vascular injury, inflammation, degeneration, and thrombosis **(Singh, 2002 and Weber and Noels, 2011).**

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).**

The first paper regarding ischemic cerebrovascular disease and *H. pylori* was published in 1997 **(Danesh et al., 1997).** It was further supported by other studies from developing and developed countries **(Markus and Mendall, 1998; Annuziata et al., 2003; Zade et al, 2009 and Park et al., 2011)**. More recent study from Iran **(Vafaeimanesh et al. 2013)** documented the association between *H. pylori* infection and CAD. The study involved 62 CAD patients and 58 controls. *Helicobacter pylori* was more prevalent among patients with CAD and with increasing the number of coronary arteries with stenosis, the *H. pylori* seropositivity increased so that 76.3% of patients with multiple vessel diseases (MVD) and 70% of patients with single vessel diseases (SVD) were *H. pylori* seropositive versus 50% in control group and this difference was statistically significant between groups (P=0.006). In Gaza strip, only two recent studies investigated *H. pylori* infection in malnutrition and type 2 diabetes medical services patients **(Abu Jabal, 2012 and Saadallah, 2013)**. The present study is the first to assess *H. pylori* Infection as a risk factor for CAD in Gaza strip.

1.2 General objective

To asses *H. pylori* infection as a risk factor for CAD in Gaza strip**.**

1.3 Specific objectives

1. To determine the prevalence of *H. pylori* infection among CAD cases compared with controls.
2. To estimate cardiac enzymes including aspartate aminotransferase (AST), lactate dehydrogenase (LDH), Creatinine phosphokinase (CK) and Creatinine phosphokinase MB (CK_{MB}) in cases and controls.

3. To measure lipid profile including cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) in cases compared to controls.
4. To evaluate blood parameters including white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb) and platelet (PLT).
5. To verify the relationship between *H. pylori* and the studied parameters in CAD patients**.**

1.4 Significance

1. Coronary artery disease becomes the first leading cause of death globally as well as among Palestinians according to Ministry of Health **(Ministry of Health, MOH, 2010)**.
2. To find out whether exposure to *H. pylori* infection is associated with CAD, this may be of a prognostic value.
3. This is the first study to assess *H. pylori* infection as a risk factor for CAD patients in Gaza strip.
4. Understanding the role of *H. pylori* in CAD could be useful in the management of the disease.

Chapter 2

Literature Review

2.1 Heart and coronary arteries

The heart is a muscular (myocardium) organ that pumps blood throughout the blood vessels to various parts of the body by repeated rhythmic contractions. The human heart is located anterior to the vertebral column and posterior to the sternum and it is enclosed in a double-walled sac called the pericardium **(Starr et al., 2009)**. The heart has four chambers, two superior atria (receiving chambers) and two inferior ventricles (discharging chambers). Oxygenated blood returns from the lungs into the left atrium, which pumps it into the left ventricle, whose subsequent strong contraction forces it out through the aorta leading to the systemic circulation **(Marieb, 2011)**. Coronary arteries arise from aorta (Figure 2.1). The right coronary artery gives branches to the walls of the right atrium and ventricle. The left coronary artery gives branches to supply the roots of the aorta and pulmonary trunk and the walls of the left atrium and ventricle. The left coronary artery bifurcates into the left anterior descending artery and the left circumflex artery.

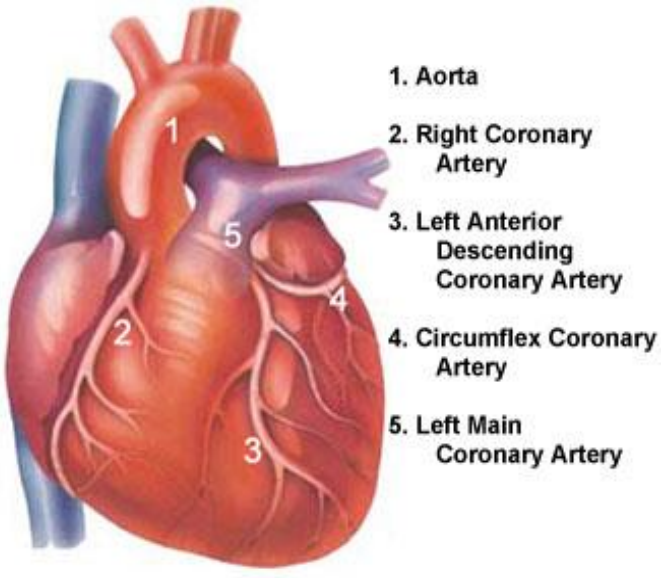


Figure 2.1. Coronary arteries of the heart **(Encyclopedia of Science, 2013)**

2.2 Definition of coronary artery disease

Coronary artery disease, also known as coronary heart disease (CHD), coronary atherosclerosis and ischemic heart disease (IHD), which is a branch of CVD and a common form of heart disease. Coronary artery disease is considered as an insidious and dangerous disease in the world, and the major source of morbidity and mortality in the developed world **(Walter, 2008 and European Society of Cardiology guideline, 2013).** It is caused by atherosclerosis, an accumulation of fatty materials in the inner linings of arteries. The resulting blockage restricts blood flow to the heart and when the blood flow is completely cut off, the result is heart attack **(CDC, 2009).**

2.3 Epidemiology of coronary artery disease

According to World Health Organization fact sheet in 2007, CVD are the number one cause of death globally, more people die annually from CVD than from any other cause. An estimated 17.5 million people died from CVD in 2005, representing 30% of all global deaths. Of these deaths, an estimated 7.6 million were due to CAD and 5.7 million were due to stroke. Over 80% of CVD deaths take place in low- and middle-income countries and occur almost equally in men and women. By 2015, almost 20 million people will die from CVD, mainly from heart disease and stroke **(World Health Organization, WHO, 2007).**

Cardiovascular diseases affect many people in middle age, very often severely limiting the income and savings of affected individuals and their families. Cardiovascular diseases place a heavy burden on the economies of countries. Lower socioeconomic groups in high-income countries generally have a greater prevalence of risk factors, diseases and mortality. A similar pattern is emerging as the CVD epidemic evolves in low and middle-income countries **(Gersh et al., 2010).**

In the United Kingdom, CVD accounted for almost 198,000 deaths in 2006. Each year CVD causes over 2 million deaths in the European Union (EU), representing 42% of all deaths in the EU **(British Heart Foundation, 2008).** Furthermore in

Canada every 7 minutes someone dies from heart disease or stroke; CVD accounted for 31% of all deaths in Canada. Moreover, estimates for the year 2006 are that 80 million people in the United States have one or more forms of cardiovascular diseases; CAD caused about half million deaths and is the single leading cause of death in America today **(AHA, 2009).**

In the Eastern Mediterranean region (Bahrain, Cyprus, Egypt, Iran, Iraq, Jordan, Kuwait, Oman, Qatar, the United Arab Emirate) CVD are emerging as a major health problem. The proportion of death from CVD ranges from 25 to 45%. Coronary artery disease seems to be the predominant type of cardiomyopathy encountered in many of these countries, and hospital data indicate rising trends. Several of these countries have experienced rapid socioeconomic changes over the last two decades. Daily caloric intake has increased **(Heart Encyclopedia, 1990).** Approximately 37% of adults reported having two or more risk factors of heart disease and stroke including high blood pressure, high serum cholesterol level, diabetes, smoking, and obesity **(Hayes et al., 2005).**

In Palestine, CVD principally heart disease, is the first leading cause of death among Palestinians **(MOH, 2010)**. A total of 1088 cases from 3406 in Gaza strip, with proportion of 31.9%, died from CVD while mortality among males was higher than females (591 males vs 497 females, 54.3% in males vs 45.7% in females), and 1708 from 5581 with proportion of 30.6% in the west bank died from CVD while mortality among males was higher than females (966 males vs 742 females, 56.6% in males vs 43.4% in females) **(MOH, 2010)**.

2.4 Pathophysiology of coronary artery disease

Coronary artery disease is caused by coronary artery atherosclerosis which refers to the presence of atherosclerotic changes within the walls of the coronary arteries. The process of arterial narrowing with atherosclerotic plaque development is called atherogenesis which appears to be initiated and/or facilitated by chronic injury to the endothelium **(Hansson, 2005)**. Low density lipoprotein compound bounds to LDL receptor, internalized, and transported through the endothelium the oxidative

modification of LDL (Figure 2.2). The Oxidized LDL (OxLDL) contributes to atherogenesis by the following **(Stocker et al., 2004)**:-

1. Aiding the recruitment of circulating monocytes into the intimal space
2. Inhibiting the ability of resident macrophages to leave the intima
3. Enhancing the rate of uptake of the lipoprotein with leads to foam cell formation
4. Be a cytotoxic by leading to loss of endothelial integrity

Therefore, many of substantial evidences have documented that the development of CAD involves lipid oxidation and formation of oxygen radicals and that atherosclerosis with inflammation are associated with formation of oxygen and peroxyl radicals **(Tigran et al., 2005)**.

The macrophages remove OxLDL via scavenger receptors by forming the foam cells which are lipid filled macrophages **(Rocha and Libby, 2009 and Emedicine, 2013)**. The accumulated plaques of core lipid, thick fibrous filled with inflammatory cells as macrophage and T cells may be ruptured in response to the physical forces of blood flowing inside swelling of artery wall **(Stocker et al., 2004)**. Blood platelets begin to accumulate at the site of a vulnerable coronary plaque and to initiate thrombotic occlusion of the coronary vessel **(Meinrad, 2004 and Rocha and Libby, 2009)**.

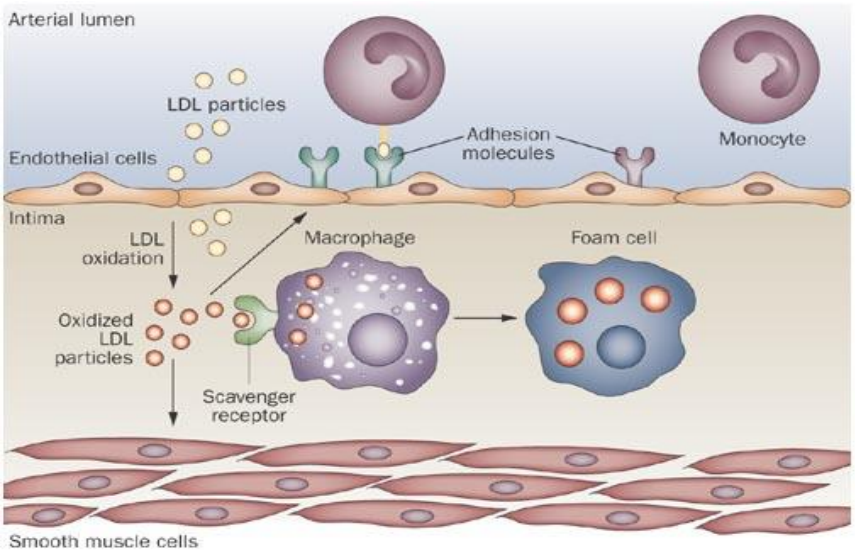


Figure 2.2. Pathophysiology of coronary artery disease **(Rocha and Libby, 2009)**

2.5 Risk factors of coronary artery disease

2.5.1 Socioeconomic status

In developing countries CAD risk factors are associated with lower level of education and income **(Gupta, 2003).** Studies conducted in other developed countries have provided convincing evidence of an inverse relationship between socioeconomic status (SES) and CAD. However, when multiple countries are compared, the relationship is quite variable, depending on the level of health transition in each country **(Reddy et al., 2007).** In addition, weighted data on self-reported CAD and SES showed that self-reported CAD prevalence was generally higher among those of lower SES **(Cunningham, 2010).** In a recent US study, socioeconomic status based on a combination of income and education was significantly associated with estimated 10-year global CAD risk in all racial/ethnic groups except foreign-born Mexican American men **(Karlamangla et al., 2010).**

2.5.2 Family history

The study conducted by **Kelalainen et al. (1996)** in Finland has revealed that the family history of CAD was positively associated with cumulative occurrence of CAD events (Odds ratio 2.53; P=0.009). In addition, a study carried out by **Sesso et al. (2001)** has revealed that family history of premature CAD is an independent risk factor for CAD events.

Nasir et al. (2004) assessed the association of a family history of premature CAD with coronary artery calcification (CAC) in 8549 asymptomatic individuals (69% men; mean age, 52±9 years) and compared the effects of sibling or parental family history on the risk of subclinical atherosclerosis. They demonstrated a highly significant association between family history of premature CAD and the presence and extent of CAC. A sibling history was more strongly associated with sub clinical coronary atherosclerosis than a parental history of premature CAD.

In a multi-Ethnic study of atherosclerosis on 5347 asymptomatic individuals (47% men; mean age 62±10 years), **Nasir et al. (2007)** showed an association between family history of premature CAD and the presence of any CAC**.** In addition, **Braekkan et al. (2008)** reported that family history of myocardial infarction remained a significant risk factor for total venous thromboembolism.

In the European prospective investigation of cancer (EPIC)-United Kingdom cohort, **Sivapalaratnam et al. (2010)** tested whether adding family history of premature CAD in first-degree relatives improves risk prediction compared with the Framingham risk score (FRS) alone. The study comprised 10 288 men and 12 553 women aged 40-79 years participating in the EPIC-Norfolk cohort who were followed for a mean of 10.9±2.1 years. A family history of CAD was indeed associated with an increased risk of future CAD, independent of established risk factors (FRS- adjusted Hazard Ratio of 1.74 (95% CI 1.56 to 1.95) for family history of premature CAD).

Hasanaj et al. (2013) assessed whether CHD risk assessment is improved when family history information is added to other clinical information recommended in guidelines. Data reveled that family history was an independent risk factor for CHD (odds ratio=1.7, 95% confidence interval=1.26–2.47) and improved discrimination beyond guideline-defined clinical factors (P<0.006). However, the difference in the area under the curve of 2.8% and the extent of patient reclassification resulting from the inclusion of family history were small (P=0.11).

2.5.3 Smoking

The impact of smoking on the risk of non-fatal acute myocardial infarction (MI) in young middle age people was studied **(Mähönen et al., 2004).** The prevalence of smoking in people aged 35–39 years who experienced non-fatal MI events was 81% in men and 77% in women. It declined with increasing age to 45% in men aged 60–64 years and 36% in women, respectively. In the 35–39 years age group the relative risk of non-fatal MI for smokers was 4.9 (95% confidence interval (CI)

3.9 to 6.1) in men and 5.3 (95% CI 3.2 to 8.7) in women, and the population attributable fractions were 65% and 55%, respectively. The authors concluded that during the study period more than half of the non-fatal MIs occurring in young middle age people can be attributed to smoking.

In developing countries about 2.41 million premature deaths from cardiovascular causes were attributed to smoking **(Hossain et al., 2007)**. In addition, **Mannan et al. (2011)** concluded that age at quitting of smoking improves the prediction of risk of CAD incidence even after other smoking measures are taken into account.

Sadeghi et al. (2013) analyzed data of 120 patients, 92 (73.6%) were male, 113 (90.4%) were married, 58 (46.4%) were smokers, 19 (15.2%) were opium users, 97 (77.6%) had dyslipidemia, 44 (35.2%) had hypertension, and 33 (26.4%) had diabetes mellitus. In addition, family history was presented in 54 patients (43.2%). Among the study population, 120 patients (96%) had at least one of the traditional risk factors, including dyslipidemia, hypertension, diabetes mellitus, cigarette smoking, and family history of CHD. However, none of the dyslipidemic patients had controlled total cholesterol, LDL-C, HDL-C, and triglyceride. Also, none of the diabetic patients had hemoglobin A1C<7%. Among the 44 hypertensive patients, blood pressure of 15 ones (34%) was within the normal range.

2.5.4 Physical activity

Wannamethee and Shaper, (1997) in United Kingdom conducted a prospective study of 5159 subjects and indicated that there was an inverse relationship between CAD rates and physical activity with lowest rates in men undertaking moderate physical activity. On the other hand, **Wannamethee et al., (2000)** reported that regular moderate physical activity, such as rapid walking or similar levels of exertion, is associated with a 30-50% reduction in the risk of CHD. In this regard United Kingdom Department of Health guidelines recommend at least 30 min of moderate activity on most days of the week will reduce the risk of CHD by 30-50% **(Bassuk and Manson 2005).**

Fransson et al. (2006) assessed whether little physical activity in leisure-time compensates for the increased risk of AMI associated with overweight and obesity. Results was associated with a decreased risk with subjects with BMI less than 30 kg\high square which means among lean, normal-weight and overweight subjects, but not among (BMI >30) obese subjects. While obese persons had an almost twofold risk of myocardial infarction even if he physically active person, compared with normal-weight and sedentary persons (OR 1.85, 95% CI 1.07-3.18). The results were similar for both genders.

Weinstein et al. (2008) revealed that higher BMI and physical inactivity were individual predictors of CHD. In addition, **Lightfoot et al. (2010)** reported that physical activity interventions increase lifespan and improved the quality of life of heart patients. Furthermore, **Stewart et al. (2013)** concluded that low physical activity was only partly explained by cardiovascular symptoms

2.5.5 Obesity

Research articles indicated that obesity independently predicts CAD. This relation appears to exist for both men and women with minimal increases in BMI. Relations between categories of BMI, CAD risk factors, and vascular disease end points were examined prospectively in Framingham Heart Study participants aged 35 to 75 years, who were followed up to 44 years **(Wilson et al., 2002).** The age-adjusted relative risk (RR) for new hypertension was highly associated with overweight status (men: RR, 1.46; women: RR, 1.75). New hyper cholesterol and diabetes mellitus were less highly associated with excess adiposity. The age-adjusted RR (confidence interval [CI]) for CAD was increased among those who were overweight (men: 1.21 [1.05-1.40]; women: 1.20 [1.03-1.41]) and the obese (men: 1.46 [1.20-1.77]; women: 1.64 [1.37-1.98]). High population attributable risks were related to excess weight (BMI ≥25) for the outcomes hypertension (26% men; 28% women), angina pectoris (26% men; 22% women), and CAD (23% men; 15% women).

Shiraishi et al. (2006) examined the relation of obesity to AMI in 1260 Japanese AMI patients. In young, middle-aged, and older males, as well as in older females, cases had higher BMI than controls. Except for very old males, the prevalence of hyper cholestermia, hypertension, and diabetes mellitus were higher in each subgroup of cases than in controls. Multivariate logistic regression analysis revealed that obesity (BMI≥25) was an independent risk for AMI in young and middle aged males, but not in female. The authors concluded that obesity is significantly associated with AMI, independent of the classic coronary risk factors, in young and middle-aged males. In addition, **Yao et al. (2007)** demonstrated that BMI as a measure of overall adiposity in Chinese sample of 2334 is strongly associated with increased prevalence of CAD independent of metabolic syndrome.

In their review study entitled "Obesity and coronary artery disease: risk factor, paradox, and impact of weight loss", **Lavie et al. (2009)** reported that obesity had a major impact on CVD, such as heart failure, CAD, sudden cardiac death and atrial fibrillation, and is associated with reduced overall survival. Even in children, **Jonge et al. (2011)** pointed out that obese children had a greater left ventricular mass (1.04 SD score [95% CI: 0.20 to 1.89]) and a higher fractional shortening (0.91 SD score [95% CI: 0.02 to 1.80]).

2.5.6 Diabetes mellitus

A prospective study was carried out on 1059 type 2 diabetic patients aged 45-64 years in Finland to investigate the risk of lipid and glucose abnormalities and the occurrence of CAD **(Lehto, 1997).** Results showed that low HDL-C, high LDL-C (≥5.2 mmol/l), high triglyceride level (>2.3 mmol/l), and high fasting plasma glucose (>13.4 mmol) was independently associated with twofold increase in the risk of CAD, while high fasting glucose (>13.4 mmol/l) with low HDL-C, low HDL-C/TC ratio, or high total triglyceride increase the risk of CAD events to threefold. In addition, **Vilbergsson et al., (1998)** has indicated that type 2 diabetes increased twice the risk of CAD mortality in male and female patients independently of other risk factors.

A prospective study of 514 patients with unstable angina or MI in Iran has investigated that the diabetes mellitus and hypertension are leading risk factors that may directly or indirectly interfere and predict more serious complications of CAD **(Esteghamati et al., 2006).** Another prospective study from Japan on 13355 men and 15724 women who reported diabetes in a questionnaire has revealed that diabetes increases the risk of mortality from CAD among men and that from cancer among women **(Oba et al., 2008).**

Borg et al., (2010) examined the association between various indices of glycaemia measured during everyday activities and metabolic CAD risk factors in the A1C-Derived Average Glucose study. Participants (268 with type 1 diabetes, 159 with type 2 diabetes) completed 16 weeks of intensive continuous glucose monitoring and self-monitoring of blood glucose. For both diabetes types, the overall strongest associations with CAD risk factors were seen for the measures of average glycaemia (mean blood glucose and HbA1c). Associations between self-monitored postprandial and fasting glucose and CAD risk factors were weaker, but significant. Measurements of blood glucose variability showed non-significant associations.

Assareh et al. (2013) conducted a cross-sectional study on 333 patients who were admitted with diagnosis as a CAD and candidates for angioplasty in Iran. The prevalence of CAD risk factors in overall subjects were: 45.3% hypertension (considering >140/90 mmHg); 34.5% high cholesterol (>240 mg/dl); 27.6% diabetic mellitus (>126 mg/dL); family history of heart disease: 20.7%; smokers: 19.9%.

2.5.7 Hypertension

Many research articles about hypertension were carried out. Some discussing its impact on individual and community, others discussing the prevalence and its economic burden, in addition of studying its relationship with other CAD and other chronic disorders. A prospective cohort study in the Netherlands has indicated that there is a strong positive relationship between the occurrence of CAD and the elevated systolic and diastolic blood pressure **(van den et al., 1996)**

Demosthenes et al. (2005) evaluated the association of blood pressure measurements with CAD mortality among 12763 men, aged 40 to 59 years, from 7 countries (United States, Japan, Italy, Greece, former Yugoslavia, Finland, and the Netherlands). All baseline blood pressure measurements were the best predictors of CAD mortality; compared with age, physical activity, total cholesterol, BMI, and smoking. Moreover, pulse pressure and diastolic and systolic blood pressures were the best predictors for CAD death, followed by mean and mid blood pressures. The age-adjusted hazard ratio (HR) per 10-mm Hg increase in pulse pressure varied among cohorts from 1.19 in the United States (P=0.04) to 1.29 in southern Europe (P=0.01). Differences among cohorts were not significant. In the pooled cohorts, pulse pressure measurements were also a significant predictor for CHD (HR per 10-mm Hg increase, 1.15; P=0.04) as well as stroke death (HR per 10-mm Hg increase, 1.32; P=0.01).

Dorjgochoo et al. (2009) determined whether various levels of blood pressure, particularly normal and high normal blood pressure or prehypertension can predict cardiovascular mortality among 68438 urban Chinese women. The authors identified 1574 deaths from all causes, 247 from stroke, and 91 from CHD. Hypertension and higher levels of individual blood pressure parameters including systolic blood pressure, diastolic blood pressure, pulse pressure, and mean arterial pressure, were positively associated with all-cause, stroke, and CHD mortality (P trend <0.05 for all except for diastolic blood pressure and CHD mortality). Prehypertension (adjusted hazard ratio (HR adj) =1.65; 95% CI, 0.98 2.78), particularly high normal blood pressure (HR adj =2.34; 95% CI, 1.32–4.12), was associated with an increased risk of mortality from stroke. Hypertension accounted for 9.3% of mortality from all causes, 25.5% of mortality from stroke, and 21.7% mortality from CHD. High normal blood pressure accounted for 10.8% of mortality from stroke. Isolated systolic blood pressure also predicted stroke and CHD mortality.

Data on blood pressure collected from Canadian adults at 15 sites from March 2007 through February 2009 showed that the prevalence of hypertension in adults was estimated at 19% **(Kathryn et al., 2010).** The authors emphasized that high

blood pressure is a major risk factor for heart and vascular disease and is an important cause of death around the world. In addition, **CDC morbidity and mortality weekly report (2011)** considered hypertension as a major modifiable risk factor for CVD that affects one in three adults in the USA and contributes to one out of every seven deaths and nearly half of all CVD–related deaths in the US.

Oladapo et al. 2013 identified the relationship between CV risk factors and CVD. Commonest source of medical information was the family/friend/opinion leaders of trusted groups in 1198 (59.9%), the media (including radio, public enlightenment programmes, and newspapers) in 492 (24.6%), and the doctor/nurse/health worker in 183 (9.1%) of the respondents. About 56% of the respondents could not identify a single risk factor. They found that subjects who had more years of formal education, a positive family history of CVD, and self reported history of diabetes mellitus were more likely to have a good level of knowledge of hypertension and other CV risks when adjusted for age, gender and marital status.

2.5.8 Lipid abnormalities

A study enrolled 147 patients with CAD (97 men and 50 women, aged 25-82 years) revealed that higher levels of remnant lipoproteins in fasting serum predict future coronary events in patients with CAD independently of other risk factors **(Kugiyama et al., 1999).** Patients with diabetes have low HDL-C, high LDL-C level, but they had significant elevated triglyceride level when compared with individuals with normal glucose tolerance, an increase of 10 mg/dl in LDL-C is associated with 12% increase in CVD risk. LDL-C blood level was considered as a strong independent predictor of CAD in diabetic patients **(Howard, 2000).**

Washio et al. (2001) evaluated the effect of hypertension, dyslipidemia and diabetes mellitus on the development of coronary atherosclerosis in 433 Japanese patients (254 men and 179 women) aged 30 years or older who underwent coronary angiography for suspected or known CHD angina. The main outcome measure was angiographically defined coronary artery stenosis and was found to a

significant degree in 146 patients (33.7%). Hypertension, diabetes, low levels of HDL-C and hypertriglyceridemia remained as significant CAD risk factors even after controlling for age, sex, hospital, smoking, alcohol use, BMI and leisure time physical activity. However, hypercholesterolemia was not a significant risk factor after adjusting for these variables. After controlling for these variables, diabetes, low HDL-C and hypertriglyceridemia were significant CAD risk factors for men, but only DM was a significant CAD risk factor in women.

Pischon et al. (2005) compared apolipoprotein B (apoB), non–HDL-C, LDL-C, and other lipid markers as predictors of CHD among 18225 men. A total of 266 had nonfatal myocardial infarction or fatal CHD during 6 years of follow-up. After adjustment for matching factors, the relative risk of CHD in the highest quintile compared with the lowest quintile was 2.76 (95% confidence interval [CI], 1.66 to 4.58) for non–HDL-C, 3.01 (95% CI, 1.81 to 5.00) for apoB, 1.81 (95% CI, 1.12 to 2.93) for LDL-C, 0.31 (95% CI, 0.18 to 0.52) for HDL-C, 2.41 (95% CI, 1.43 to 4.07) for triglycerides (all P trend <0.001), and 1.42 (95% CI, 0.86 to 2.32, P trend =0.19) for lipoprotein(a). When non–HDL-C and LDL-C were mutually adjusted, only non–HDL-C was predictive of CHD. When non–HDL-C and apoB were mutually adjusted, only apoB was predictive; the relative risk was 4.18 (95% CI, 1.30 to 13.49; P trend =0.02) for apoB compared with 0.70 (95% CI, 0.21 to 2.27; P trend =0.72) for non–HDL-C. Triglycerides added significant information to non–HDL-C but not to apoB for CHD risk prediction.

Associations between HDL-C and both stroke and CHD in the Asia Pacific region was investigated **(Woodward et al., 2007).** After adjustment for age and regression dilution, hazard ratios (95% confidence intervals) for a 1 standard deviation (SD) lower level of HDL cholesterol (0.4 mmol/L) were: for CHD events, 1.39 (1.22–1.57); for ischemic stroke, 0.90 (0.75–1.07), and for haemorrhagic stroke, 0.89 (0.74–1.07). As total cholesterol (TC) increased relative to HDL-c, the risk of CHD increased, the risk of ischemic stroke was unchanged but the risk of haemorrhagic stroke decreased. A 1 SD increase in TC/HDL cholesterol (1.63 units) was associated with a 27% decrease in the risk of haemorrhagic stroke (95% confidence interval, 7– 44%).

Di angilantonio et al. (2009) assessed major lipids and apolipoproteins in vascular risk among 302 430 people from Europe and North America. The rates of CHD per 1000 person-years in the bottom and top thirds of baseline lipid distributions, respectively, were 2.6 and 6.2 with triglyceride, 6.4 and 2.4 with HDL-C, and 2.3 and 6.7 with non–HDL-C. Adjusted HRs for CHD were 0.99 (95% CI, 0.94-1.05) with triglyceride, 0.78 (95% CI, 0.74-0.82) with HDL-C, and 1.50 (95% CI, 1.39-1.61) with non–HDL-C. Hazard ratio were at least as strong in participants who did not fast as in those who did. The HR for CHD was 0.35 (95% CI, 0.30-0.42) with a combination of 80 mg/dL lower non–HDL-C and 15 mg/dL higher HDL-C. For the subset with apolipoproteins or directly measured LDL-C, HRs were 1.50 (95% CI, 1.38-1.62) with the ratio non–HDL-C/HDL-C, 1.49 (95% CI, 1.39- 1.60) with the ratio apo B/apo AI, 1.42 (95% CI, 1.06-1.91) with non–HDL-C, and 1.38 (95% CI, 1.09-1.73) with directly measured LDL-C. Hazard ratios for ischemic stroke were 1.02 (95% CI, 0.94-1.11) with triglyceride, 0.93 (95% CI, 0.84-1.02) with HDL-C, and 1.12 (95% CI, 1.04-1.20) with non–HDL-C.

In his study entitled " Impact of Hypertriglyceridemia on Endothelial Dysfunction During Statin ± Ezetimibe Therapy in Patients With Coronary Heart Disease" **Nakamura et al. (2011)** concluded that hypertriglyceridemia is independently associated with endothelial dysfunction in patients with CHD during statin therapy. Ezetimibe add-on therapy improves endothelial function in these high-risk populations.

Tripta and Sharma, (2013) evaluated the association of BMI, blood lipids and apolipoproteins level with CAD among 150 CAD patients aged 35-45 years; One hundred fifty normal controls were involved. The study demonstrated that CAD patients had elevated BMI in both males and females than normal controls. Apo B levels were an important predictor of CAD. ApoA/ApoB ratio among CAD patients was 0.74 compared with 1.53 in normal subjects; controls had 105.8% higher ApoA/ApoB ratio than CAD subjects. Total cholesterol, LDL-C, triglycerides, LDL-C/HDL-C ratio of the two groups also showed significant differences. Prevalence of obesity in CAD patients was 70.7% compared with 10% in normal controls.

2.6 *Helicobacter pylori*

2.6.1 Definition and general characteristic of *H. pylori*

Helicobacter pylori is a spiral or slightly curved Gram negative rod with 2-6 characteristic unipolar flagella (Figure 2.3). The bacterium has bluntly rounded ends and measures 2.5-4.0 µm in length and 0.5-1.0 µm in width. The cell wall is smooth and may be coated with a prominent glycocalyx with a thickness of up to 40 nm **(Goodwin et al., 1989)**. The flagella measure 2.5 µm in length and around 30 nm in thickness, and have a distinctive terminal bulb **(Goodwin & Worsley, 1993)**. The bacterium displays remarkable motility in viscous solutions, and the flagella play a central role in this motility **(Hazell et al., 1986 and Suerbaum et al., 1993)**. *Helicobacter pylori* is a microaerophilic and under certain circumstances it can be U- shaped or coccoid **(Enroth et al., 1999)**. It resides naturally in the gastrointestinal tract of humans and animals **(Fox, 2002)**. In the stomach, the majority of *H. pylori* can be found in the gastric mucosa; however a few are found adhered to the gastric mucosal epithelium. The bacterium is highly adapted to survive in the hostile environment of the stomach where few other organisms can survive. Although *H. pylori* is considered to be an extra cellular bacteria, there is evidence suggesting that the bacteria has a mechanism for intracellular invasion **(Kusters et al., 2006)**.

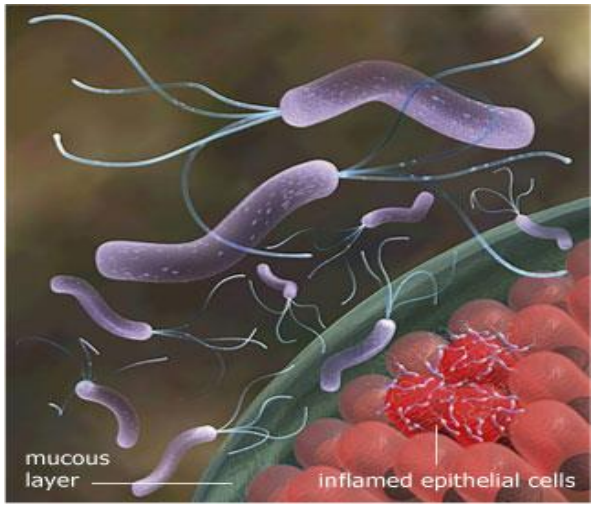


Figure 2.3 *Helicobacter pylori*. The curved bacillus with unipolar flagella is visualized by a scanning electron microscope **(Lembo, 2005).**

2.6.2 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*

2.6.3 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 in more than 70% in the populations **(Nurgalieva et al., 2002 and Stasi et al., 2008)**. On the contrary, in developed countries, *H. pylori* infection is less common in young children and increases with age and reaches 50% by adulthood **(Lane et al., 2006 and Zhou et al., 2012)**. In the Gaza strip, Abu-Mughesieb study showed that the rate of *H. pylori* infection in Gaza strip is 48.3% **(Abu-Mughesieb, 2007)**. In a recent study focused on *H. pylori* infection and malnutrition, **Abu Jabal (2012)** reported 70.5% prevalence of *H. pylori* among type 2 diabetic medical services patients in Gaza strip**.**

2.6.4 Transmission

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 has been detected in the saliva of *H. pylori* positive subjects by polymerase chain reaction (PCR) **(Khalifa et al., 2010 and Collazo, 2012)**. *Helicobacter 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 patients **(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).**

2.6.5 Signs and symptoms of *H. pylori* infection

Most people with *H. pylori* infection are asymptomatic, but a proportion of infected individuals develop severe gastro duodenal disease, including duodenal ulcer, gastric ulcer, gastric adenocarcinoma and gastric Mucosa Associated Lymphoid Tissue (MALT) lymphoma **(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)**.

2.6.6 Diagnosis of *H. pylori* infection

Diagnosis of *H. pylori* 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 *Helicobacter 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)**.

2.6.7 Pathogenic mechanisms of *H. pylori* which predispose to coronary artery disease

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 CAD, 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 that may induce or accelerate atherosclerosis **(Yamaoka et al., 2002).** Chronic infection of *H. pylori* increase the production of various metabolites, such as inflammatory cytokines, that affect the blood flow in vessels and cause endothelial dysfunction and further shrinkage of small vessels **(Tsai et al., 2001 and Annuziata et al., 2003).** Elevated concentrations of cytokines in the gastric mucosa of *H. pylori* infected patients could increase serum fibrinogen and leukocytes. It seems that inflammatory response and related reactions in patients with *H. pylori* infection could justify accompaniment of this infection and acute coronary syndrome **(Zade et al, 2009).**

2.7 Related studies

Sebastián et al. (2001) investigated the presence of *H. pylori* in 38 atherosclerotic plaques obtained at carotid endarterectomy by using morphological and immunohistochemical (intercellular adhesion molecule-1) techniques and a highly sensitive PCR method. They also examined 7 carotid arteries obtained at autopsy from subjects without carotid atherosclerosis. *Helicobacter pylori* DNA was found in 20 of 38 atherosclerotic plaques. Ten of the *H. pylori* DNA–positive plaques also showed morphological and immunohistochemical evidence of *H. pylori* infection. None of 7 normal carotid arteries was positive for *H. pylori*. Intercellular adhesion molecule-1 was expressed in 75% of *H. pylori*–positive plaques and in 22% of *H. pylori*–negative plaques. The presence of the microorganism was associated with male sex but was independent of age, vascular risk factor profile, and prior neurological symptoms.

The relationship between *H. pylori* infection and acute ischemic stroke in 62 patients with their first stroke and 143 controls was investigated **(Sawayama et al., 2005)**. All patients underwent cranial CT scanning and/or brain magnetic resonance imaging, duplex ultrasonography of the extracranial carotid arteries, and transthoracic echocardiography. *Helicobacter pylori* infection was diagnosed by detection of anti-*H. pylori* IgG antibodies, the 13C-urea breath test, and histology. Chronic *H. pylori* infection was associated with a higher risk of stroke due to small artery occlusion (odds ratio: 9.68; 95% CI: 3.56–33.08, $P < 0.001$) and a lower risk of cardioembolic stroke (odds ratio: 0.27; 95% CI: 0.03–1.53). Chronic *H. pylori* infection still showed an overall association with ischemic stroke (odds ratio for all subtypes combined: 2.57; 95% CI: 1.09–6.08) after adjusting for major cardiovascular risk factors.

Miyazaki et al. (2006) examined whether *H. pylori* infection is a risk factor in 33 patients with acute coronary syndromes (ACS). A control group was consisted of 66 males who had normal resting electrocardiogram and had no history of IHD. *Helicobacter pylori* seropositivity was determined by an IgG-specific ELISA. The presence of antibodies specific to the antigen CagA of *H. pylori* was confirmed, using CagA ELISA. Seropositive rate of IgG antibodies in patients with ACS was

87.9%. A rate in controls was 66.7%. After adjustment for age, a statistically significant association was found in *H. pylori* seropositivity between ACS and controls (OR, 3.74; 95% CI, 1.15–12.13). This relation was also significant after adjusted for potential confounding factors (OR, 4.09; 95% CI, 1.10–15.17). Anti-CagA positive *H. pylori* were significantly recognized in ACS (adjusted OR, 3.58; 95% CI, and 1.08–11.82). However, this significant association was disappeared after adjusted for potential confounding factors (P= 0.054).

The seroprevalence of *H. pylori* in patients with CAD was determined **(Veev et al., 2007)**. Patients with CAD (n=90) and control group (n=90) were enrolled into randomized, multi-centre study. Coronary artery disease risk factors analyzed included age, male gender, diabetes mellitus, systemic hypertension, cigarette smoking, hypercholesterolemia and socioeconomic status. The results showed a higher seroprevalence of *H. pylori* infection in patients with CAD compared to controls (78.8% vs. 58.3%, P<0.05). However, *H. pylori* seropositivity was not associated with coronary artery risk factors (smoking, body mass index, diabetes mellitus, hypertension, total cholesterol and socioeconomic status) either in the whole study population or in the patients and control subjects analyzed separately (P>0.05).

Nozari et al. (2009) investigated whether *H. pylori* infection is related to prevalence of CHD. The study was carried out on 130 subjects who underwent coronary angiography. According to angiography findings, the patients were grouped into cases (n=70) with CAD, and normal control group (n=60). Then, using ELISA method, specific anti *H. pylori* IgGs were measured in all subjects. Among the 130 patients, anti-*H. pylori* IgG were detected in 80% of cases and 65% of control subjects (P=0.05). The investigation showed that CAD correlated significantly with hypertension, diabetes, and smoking (P<0.05) although there was no associations between these traditional risk factors, and *H. pylori* infection.

The relationship between *H. pylori* and MI was determined **(Azarkar et al. 2011).** Seventy-three MI patients and 78 individuals with no history of this disease were

compared. Patients and controls were matched for age and sex. Levels of serum IgA and IgG antibodies against *H. pylori* were measured by Elisa method. The percentage of IgG positive cases against *H. pylori* was 57.5% in the case group and 32.1% in the control group (P=0.002, OR: 2.87 CI: 95%; 1.5-5.6). Meanwhile, there was no significant difference in IgA positive cases between the two groups (42.5% and 48.7% in the case and control groups, respectively) (P=0.44; OR: 0.78 95% CI; 0.41-1.48). The study showed 74.2% of cases in the case group and 45.2% in the control group were positive for both IgG and IgA (P=0.01; OR: 3.5 95% CI; 1.3-9.5). No significant differences were found between two groups in terms of relation between *H. pylori* related antibodies level and heart disease classic risk factors (smoking, hypertension,…), sex, and age, but between dyslipidemia and *H. pylori* related antibodies was significant differences in case group (P=0.05).

Tewari et al. (2012) conducted a case control study (retrospective) over a two-year period. The study population was divided into two groups with 200 individuals in each group. The first group comprised cases of CAD and the second comprised healthy controls selected from the general population after matching for age and sex. ELISA was done for immunoglobulin (IgG) antibodies to *H. pylori, Chlamydia pneumoniae (C. pneumonia)*, and *Cytomegalovirus (*CMV*).* Seropositivity for *H. pylori* was present in 119 patients of CAD (59.5%) but it was present in only 76 controls (38%) (P= 0.001). There was a statistically significant association between seropositivity for *H. pylori* and CAD. There was no statistically significant association between *C. pneumonia* and CMV seropositivity with CAD. Multiple logistic regression analysis was done with CAD as the outcome (dependent variable). The predictor covariates (independent) variables were seropositivity to *H. pylori, C. pneumoniae,* and CMV*,* hypertension, obesity, diabetes, and dyslipidaemia. It was found that seropositivity to *H. pylori*, hypertension, obesity, and dyslipidaemia were significant risk factors for CAD.

Vafaeimanesh et al. (2013) evaluated the association between *H. pylori* infection and CAD. The study involved 62 CAD patients and 58 controls. *Helicobacter pylori* was more prevalent among patients with CAD and with increasing the number of coronary arteries with stenosis, the *H. pylori* seropositivity increased so that 76.3% of patients with multiple vessel diseases (MVD) and 70% of patients with single vessel diseases (SVD) were *H. pylori* seropositive versus 50% in control group and this difference was statistically significant between groups (OR=3.86, 95%CI=1.48-10; P=0.006). Positive CAD was significantly associated with HDL level (OR=0.92, 95%CI=0.86-0.96; P=0.01) and erythrocyte sedimentation rate (OR=1.07, 95%CI=1.02-1.13; P=0.006). Also, CAD positive patients had higher C-reactive protein levels than controls and it was statistically different between SVD group versus controls (P<0.05).

Chapter 3

Materials and Methods

3.1 Study Design

Case control study design.

3.2 Study population

The study population included CAD patients (cases) aged 40-65 years attending cardiology units at Al-Shifa and Naser hospitals, Gaza Strip. Controls were apparently healthy non CAD individuals.

3.3 Sampling and sample size

Coronary artery disease patients were selected from cardiac units at Al-Shifa and Naser hospitals, Gaza Strip. Control healthy individuals with no history of CAD were selected from the general population. Cases and controls were matched for age and gender. (The sample size calculations were calculated based on the formula for case-control studies). EPI-INFO statistical package version 3.5.1 (EPI-INFO, 2008) was used with 95% CI, 80% power and 50% proportion as conservative and OR > 2. (The sample size in cases of 1:1 ratio of case control was found to be 56:56). For a no-response expectation, the sample size was increased to 62 patients. The controls also consisted of 62 healthy individuals.

3.4 Exclusion criteria

1. Cancer patients.

3.5 Ethical Considerations

An official letter of request sent from Ministry of Health to Al-Shifa and Naser hospitals, administration to facilitate the conduction of the study **(Annex 1)**.In addition, The necessary approval to conduct the study was obtained from Helsinki committee in the Gaza Strip **(Annex 2)**. Helsinki committee is an authorized professional body for giving permission to researchers to conduct their studies with ethical concern in the area.

3.6 Data collection

3.6.1 Questionnaire interview

A meeting interview was used for filling in a questionnaire which designed for matching the study need for both cases and controls **(Annex 3)**. All interviews were conducted face to face by the researcher himself. During the survey the interviewer explained any of the questions that were not clear. The questionnaire was based on the questions of a previous study with some modifications **(Asfeldt 2009, and Abu Sedo, 2012).** 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 Cardiology, Microbiology and Public Health. The questionnaire was piloted with 8 patients not included in the study. The questionnaire included questions on the personal profile of the study population (Age, gender and education); socioeconomic data (employment, family income/month, family history of CAD and smoking); physical activity, diet and compliance of medication; diabetes, hypertension, gastritis and peptic ulcer among the study population.

3.6.2 Body mass index

Body mass index was calculated as the ratio of body weight in Kg/height in square meter. Patients were asked to remove heavy clothes and shoes before measurement of weight and height. Medical balance (Seca Model 762, Germany) was used for weight measurement. People with BMI=18.5-24.9 were considered to

have normal weight, people with BMI=25.0-29.9 were classified overweight, and people with BMI≥30.0 were considered obese **(WHO, 2012).**

3.6.3 Specimen collection and biochemical analysis

Twelve hours fasting overnight venous blood samples were collected from 62 CAD patients and 62 healthy controls. Blood samples (6 ml each) were drawn by a well trained nurse into vacutainer and plastic tubes from each control and CAD patients. About 2 ml blood was placed into EDTA vacutainer tube to perform CBC for cases and controls. The remainder quantity of blood (4 ml) was placed in plastic tube and was left for a while without anticoagulant to allow blood to clot. Serum samples were obtained by centrifugation at 3000 rpm for 10 minutes for determination of AST, LDH, CK, CK_{MB}, cholesterol, triglycerides, HDL-C and LDL-C. *H. pylori* IgG was determent in serum by enzyme immunoassay (ELISA) kit. (monobind ELISA kit was perches).

3.7 Biochemical analysis

3.7.1 Detection of *H. pylori* IgG

Serum *H. pylori* IgG was determent by competitive ELISA for the quantitative determination of *H. pylori* IgG in human serum Catalog number 1425-300 IgG Size: 96 wells, monobind, United states of america (USA).

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:

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

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

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

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

k_a = Rate Constant of Association

k_{-a} = Rate Constant of Disassociation

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

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

$Streptavidin_{CW}$ = Streptavidin immobilized on well

Immobilized complex = sandwich complex bound to the solid surface

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

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

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

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

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

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

Reagents

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

Five 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 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 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 vial of serum diluent containing buffer salts and a dye. Store at 2-8°C.

F. Wash Solution Concentrate – 20ml

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

G. Substrate A – 7ml/vial

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

H. Substrate B – 7ml/vial

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

I. Stop Solution – 8ml/vial

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

Specimen collection and preparation

The specimen was blood; serum or plasma in type and the usual precautions in the collection of venipuncture samples were observed. For accurate comparison to established normal values, a fasting morning serum sample was obtained. The

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

Test procedure

Before proceeding with the assay, all reagents, serum references and controls were brought to room temperature (20-27°C).

1. The microplates wells were formatted for each serum reference, control and patient specimen to be assayed in duplicate.
2. We pipetted 25µl of the appropriate serum reference, control or diluted patient specimen into the assigned well for IgG determination.
3. We add 100µl of *H. pylori* Biotin Reagent Solution.
4. We swirled the microplate gently for 20-30 seconds to mix and cover.
5. We incubated 60 minutes at room temperature**.**
6. We discarded the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. We add 350µl of wash buffer, decant (blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes.
8. We added 100µl of *H. pylori* Enzyme Reagent to all wells.
9. We cover and incubate for thirty (30) minutes at room temperature.
10. We repeated steps (6 & 7) as explained above. Add 100µl of Working Substrate Solution to all wells.
11. We incubated at room temperature for 15 minutes.
12. We add 50µl of stop solution to each well and swirl the microplate gently for 15-20 seconds to mix.
13. We readied the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader MindrayMR-96A.

Calculation of results

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

1. We recorded the absorbance obtained from the printout of the microplate.

2. We ploted 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. We drawed the best-fit curve through the plotted points.

4. We determined the level of anti-*H. pylori* activity for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration in U/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example the average absorbance 1.603 intersects the dose response curve at 64.0 U/ml anti-*H. pylori* concentration.

3.7.2 Determination of serum aspartate aminotransferase activity

Serum AST activity was measured by using optimized UV test according to international federation of clinical chemistry and laboratory medicine **(Thomas 1998)**, using Diasys reagent Kits.

Principle

The principle of the method is based on the following enzymatic reactions:

L-Aspartate + 2-Oxoglutarate $\xleftrightarrow{AST}$ L-Glutammate + Oxalacetate

Oxalacetate + NADH + H+ $\xleftrightarrow{MDH}$ L-Malate + NAD+

Decrease in absorbance value at 340 nm, due to the oxidation of NADH to NAD+, is directly proportional to the AST activity in the sample.

Composition of reagents

Reagent	Concentration
Reagent A:	
TRIS	28 mmol/l
EDTA-Na2	5.68 mmol/l
L-Aspartate	284 mmol/l
MDH	≥ 800 U/l
Sodium azide	2 g /l
Reagent B:	
2-Oxoglutarato	68 mmol/l
NADH	1.12 mmol/l
Sodium azide	0.095 g/l

Preparation of reagents

Bireagent procedure. The reagents are liquids ready to use.

Monoreagent procedure. Ten parts of Reagent A and one part of Reagent B to obtain the working reagent (ex. 20 ml of RA + 2 ml of RB).

Analytical procedure

About 0.5 ml of serum was transferred to the Mindray BS-300 chemistry auto analyzer to perform the test according to these parameters:

Parameter	Value
Reagent (µl)	200
Serum (µl)	20
Incubation period (s)	15 cycle (3.5minutes)
Reaction type	Kinetic
Wavelength (nm)	340
Reaction	Descending

Reference value

Male:	10-50 U/l
Female:	10-35 U/l

3.7.3 Determination of serum lactate dehydrogenase activity

Serum LDH activity was measured by using optimized UV test through decrease of the absorbance value at 340 nm, due to the NADH oxidation in NAD+, is directly proportional to the enzyme activity (**Deutsche Gesellschaft für klinische Chemie, DGKC, 1972)** using Diasys reagent Kits.

Principle

Optimized method according to DGKC. LDH catalizes the following reaction:

$$\text{Pyruvate} + \text{NADH} + \text{H+} \xrightarrow{\text{LDH}} \text{L-Lactate} + \text{NAD+}$$

Decrease of the absorbance value at 340 nm, due to the NADH oxidation in NAD+, is directly proportional to the enzyme activity.

Composition of reagents

Reagent	**Concentration**
Reagent A:	
Tris buffer pH 7.5	64 mmol/l
Pyruvate	0.81 mmol/l
Sodium azide	0.095 g/l
Reagent B:	
Good buffer pH 9.6	15 mmol/l
NADH	1.05 mmol/l
Sodium azide	0.095 g/l

Preparation of the reagents

Bireagent procedure the reagents are liquids ready to use.

Monoreagent procedure ten parts of Reagent A and one part of Reagent B were mixed to obtain the working reagent (e.g.: 20 ml of RA + 2 ml of RB).

Analytical procedure

About 0.5 ml of serum was transferred to the Mindray BS-300 chemistry auto analyzer to perform the test according to these parameters:

Parameter	Value
Reagent (µl)	250
Serum (µl)	4
Incubation period (s)	15 cycles (3.5minutes)
Reaction type	Kinetic
Wavelength (nm)	340
Reaction	Descending

Calculation of results

- Activity (U/l) = ΔA/min x factor (f) indicated in the following table:

Monoreagent procedure

340 nm f = 8095

334 nm f = 8252

365 nm f = 15000

Reference values

Adults: < 480 U/l

3.7.4 Determination of serum Creatine kinase activity

Serum CK activity was measured by using optimized UV test according to recommendations of the Enzyme Commission **(German Society for Clinical Chemistry, 1977)**, using Diasys reagent Kits.

Principle

The CK activity is measured by the increasing rate of absorbance resulting from the following reactions:

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK (AMP, NAC)}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{Glucose} \xrightarrow{\text{HK}} \text{ADP} + \text{G6P}$$

$$\text{G6P} + \text{NADP+} + \text{H2O} \xrightarrow{\text{G6P-DH}} \text{Gluconate-6P} + \text{NADPH} + \text{H+}$$

Composition of reagents

Reagent	Concentration
Reagent A:	
Imidazol buffer, pH 6.7	100mmol/l
N-acetyl cysteine (NAC) 20 mmol/l	20 mmol/l
Magnesium acetate	10 mmol/l
Glucose 20 mmol/l	20 mmol/l
HK ≥ 4 KU/l	≥ 4 KU/l
Reagent B:	
Creatine phosphate 30 mmol/l	30 mmol/l
AMP	5 mmol/l
ADP	2 mmol/l
Di(adenosine-5') pentaphosphate	10 µmol/l
G6P-DH	≥ 1.5 KU/l

Preparation of the reagents

Bireagent procedure the reagents are liquids ready to use.

Monoreagent procedure four parts of Reagent A and one part of Reagent B were mixed to obtain the working reagent (e.g.: 20 ml of RA + 5 ml of RB).

Analytical procedure

About 0.5 ml of serum was transferred to the Mindray BS-300 chemistry auto analyzer to perform the test according to these parameters:

Parameter	Value
Reagent (µl)	200
Serum (µl)	10
Incubation period (s)	15 cycles (3.5minutes)
Reaction type	Kinetic
Wavelength (nm)	340
Reaction	Ascending

Calculation of results

CK activity (U/l) = ΔA/min x 4127 (37 °C)

Reference value

CK Male	≤ 174 U/l
CK Female	≤ 140 U/l
Children	≤ 225 U/l

3.7.5 Determination of serum creatine kinase MB activity

Serum CK_{MB} activity was measured by using optimized UV test according to recommendations of the Enzyme Commission **(German Society for Clinical Chemistry, 1977),** using Diasys reagent Kits.

Principle

The immunoinhibition from a specific antibody of both, MM subunits and the single M subunit of CK_{MB}, allows the determination of the B subunit. The CK_B activity, corresponding to half of CK_{MB}, is measured by the increasing rate of absorbance resulting from the following reactions:

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow[\text{HK}]{\text{CK (AMP, NAC)}} \text{Creatine} + \text{ATP}$$

ATP + Glucose →ADP + G6P

G6P-DH

G6P + NADP+ + H2O →Gluconate-6P + NADPH + H+

Composition of reagentS

Reagent	**Concentration**
Reagent A:	
Imidazol buffer, pH 6.7	100mmol/l
N-acetyl cysteine (NAC) 20 mmol/l	20 mmol/l
Magnesium acetate	10 mmol/l
Glucose 20 mmol/l	20 mmol/l
HK	≥ 4 KU/l
NADP	2.5 mmol/l
Reagent B:	
Creatine phosphate 30 mmol/l	30 mmol/l
AMP	5 mmol/l
ADP	2 mmol/l
Di(adenosine-5') pentaphosphate	10 µmol/l
G6P-DH	≥ 1.5 KU/l
Sufficient CK-M human antibody to inhibit	≥ 3000 U/l of CK-MM at 37 °C.

Preparation of the reagents

Bireagent procedure The reagents are liquids ready to use.

Monoreagent procedure Four parts of Reagent A and one part of Reagent B were mixed to obtain the working reagent (e.g.: 20 ml of RA + 5 ml of RB).

Analytical procedure

About 0.5 ml of serum was transferred to the Mindray BS-300 chemistry auto analyzer to perform the test according to these parameters:

Parameter	**Value**
Reagent (µl)	200
Serum (µl)	10
Incubation period (s)	15 cycles (3.5minutes)
Reaction type	Kinetic
Wavelength (nm)	340
Reaction	Ascending

Calculation of results

$$\text{CK-B activity (U/l)} = \Delta A/\text{min} \times 4127\ (37\ ^\circ C)$$

$$CK_{MB}\ \text{activity (U/l)} = \text{CK-B activity} \times 2$$

Reference values

$$CK_{MB}, \text{ adults: } 2.0\ \text{U/l} \div 19.5\ \text{U/l (at } 37\ ^\circ C)$$

Newborns, infants, and children have higher serum CK-MB values than adults.

A ratio between CK_{MB} and total CK activities above 4% should be considered suspicious, and above 10% consistent with acute myocardial infarction.

3.7.6 Determination of serum cholesterol

Serum cholesterol was determined by enzymatic colorimetric method for the quantitative determination of total Cholesterol in serum or plasma **(Meiatlini et al, 1978)**, using Diasys Diagnostic Systems, Germany.

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.

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

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

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

Composition of reagents

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

Analytical procedure

About 0.5 ml of serum was transferred to the Mindray BS-300 chemistry auto analyzer to perform the test according to these parameters:

Parameter	**Value**
Reagent (µl)	300
Serum (µl)	3
Incubation period (s)	20 cycles (4 minutes)
Reaction type	End point
Wavelength (nm)	500
Reaction	Ascending

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

3.7.7 Determination of serum triglyceride

Serum triglceride was determined by enzymatic colorimetric method for the quantitative determination of triglyceride in serum or plasma **(Bucolo and David, 1973)**, using Diasys Diagnostic Systems, Germany.

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.

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

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

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

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

Composition of reagents

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

Procedure

About 0.5 ml of serum was transferred to the Mindray BS-300 chemistry auto analyzer to perform the test according to these parameters:

Parameter	Value
Reagent (µl)	300
Serum (µl)	3
Incubation period (s)	20 cycles (4 minutes)
Reaction type	End point
Wavelength (nm)	500
Reaction	Ascending

Calculation

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

Reference value

Child (desirable)	30 - 150 mg/dl
Adult (desirable) Male Female	40 - 160 mg/dl 35 - 135 mg/dl

3.7.8 Determination of serum high density lipoprotein cholesterol

Liquid HDL-C precipitant for the determination of HDL-C was applied **(Grove, 1979)**, using Diasys Diagnostic Systems, Germany.

Principle

Chylomicrons, VLDL-C and LDL-C are 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.

Composition of reagentS

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

Analytical procedure

1- Precipitation

Two hundred micro liter of standard (sample or control) was 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.

Ten micro liter of the supernatant of standard (sample or control) was added to 1 ml of the cholesterol reagent and mixed well.

The mixture was incubated for 5 min at 37 ºC.

The absorbance was measured within 45 min.

Calculation

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

Reference values

Child	37 – 75 mg/dl
Adult: Male Female	35 – 65 mg/dl 35 – 80 mg/dl

3.7.9 Determination of serum low density lipoprotein cholesterol

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

Principle

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

The Equation

LDL-C = Total Cholesterol – (HDL-C) - TG/5

3.8 Hematological parameters

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

3.9 Statistical analysis

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

- Simple distribution of the study variables and the cross tabulation were applied.
- Chi-square (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 AST enzyme.
- 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.

Chapter 4

Results

4.1 Personal profile of the study population

The present study is a case control design. The study population comprised 62 apparently healthy controls (31 males and 31 females) and 62 CAD patients which represent cases (31 males and 31 females). Table 4.1 illustrates personal profile of the study population. Age classification showed that 15 (24.2%) controls and 14 (22.6%) cases were ≤45 years old. Age group 46-55 years comprised 25 (40.3%) controls and 23 (37.1%) cases. Controls and cases aged >55 years old were 22 (35.5%) and 25 (40.3%), respectively. The difference between controls and cases in term of age distribution was not significant (χ^2=0.309, P=0.857). The mean ages of controls and cases were 52.8±7.3 and 53.1±7.0 years old with ranges of 41-64 and 40-65 years, respectively. The independent sample t-test also showed no significant difference between mean ages of controls and cases (t=0.402, P=0.724). Analysis of the educational status of the study population showed that 24 (38.7%) controls and 11 (17.8%) cases had a university degree, 12 (19.4%) and 8 (12.6%) finished secondary school, 13 (20.9%) and 17 (27.4%) passed preparatory school, 7 (11.3%) and 8 (12.9%) finished primary school, and 6 (9.7%) and 18 (29.0%) were illiterate. The difference between various educational levels of controls and cases was significance (χ^2 =12.229, P=0.016).

Table 4.1 Personal profile of the study population

Personal profile	Controls (n=62)		Cases (n=62)		test		p-value
	No.	%	No.	%			
Age (Year)							
≤45	15	24.2	14	22.6	χ^2	0.309	0.857
46-55	25	40.3	23	37.1			
>55	22	35.5	25	40.3			
Mean±SD	52.8±7.3		53.1±7.0		**t**	0.402	0.724
Range (min-max)	41-64		40-65				
Gender							
Male	31	50.0	31	50.0	χ^2	0.000	1.000
Female	31	50.0	31	50.0			
Education							
University	24	38.7	11	17.8	χ^2	12.229	0.016
Secondary school	12	19.4	8	12.6			
Preparatory school	13	20.9	17	27.4			
Primery school	7	11.3	8	12.9			
Illiterate	6	9.7	18	29.0			

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

4.2 Socioeconomic data of the study population

Table 4.2 provides socioeconomic data of the study population. The employed controls and cases were 37 (59.6%) and 17 (27.4%) whereas 25 (40.3%) controls and 45 (72.6%) cases were unemployed. The difference between the two groups was significant (χ^2=13.122, P=0.000). Regarding family income\month, the number of cases with low income was higher than that of controls (χ^2=12.557, P=0.002). In addition, family history revealed that 22 (35.5%) controls and 41 (66.1%) cases reported that they have family history of CAD whereas 40 (46.5%) controls and 21 (33.9%) cases did not have family history of CAD. The difference between the two groups was significant (χ^2=11.648, P=0.001) indicating that family history is associated with CAD. Smoking was found to be associated with CAD (χ^2=8.255, P=0.004).

Table 4.2 Socioeconomic data of the study population

Socioeconomic data	Controls (n=62)		Cases (n=62)		χ^2	p- value
	No.	%	No.	%		
Employment						
Yes	37	59.6	17	27.4	13.122	0.000
No	25	40.3	45	72.6		
Family income/month (NIS)*						
<1000	19	30.7	29	46.8	12.557	0.002
1000-2000	33	53.2	14	22.6		
>2000	10	16.1	19	30.6		
Family history of CAD						
Yes	22	35.5	41	66.1	11.648	0.001
No	40	46.5	21	33.9		
Smoking						
Yes	9	14.5	23	37.1	8.255	0.004
No	53	85.5	39	62.9		

* NIS: new Israeli Shekel.
P>0.05:Not significant, P<0.05:Significant.

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

Physical activity, diet and compliance of medication among the study population are illustrated in Table 4.3. The number of cases who doing exercise was 17 (27.4%) was lower than controls 31 (50%). The difference between the two group was significant (χ^2=6.662 and P=0.008). Concerning diet, the number of controls and cases who followed diet were 16 (25.8%) and 30 (48.4%), respectively (χ^2=6.774, P=0.008). Nearly half of cases 32 (51.6%) were compliance of medication.

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

Item	Controls (n=62)		Cases (n=62)		χ^2	p-value
	No.	%	No.	%		
Physical activity						
Yes	31	50.0	17	27.4	6.662	0.008
No	31	50.0	45	72.6		
Diet						
Yes	16	25.8	30	48.4	6.774	0.008
No	46	74.2	32	51.6		
Compliance of medication						
Yes	-	-	32	51.6	-	-
No	-	-	30	48.4		

P<0.05:Significant.

4.4 Diabetes mellitus and hypertension among the study population

Table 4.4 illustrates diabetes mellitus and hypertension among the study population. The number of cases who had diabetes mellitus 40 (46.5%) was significantly higher than that of controls 24 (38.7%), with χ_2=8.267 and P=0.003. In addition, 42 (67.7%) cases were reported hypertension compared to 8 (12.9%) controls (χ_2=38.742, P=0.000).

Table 4.4 Diabetes mellitus and hypertension among the study population.

Item	Controls (n=62)		Cases (n=62)		χ^2	p-value
	No.	%	No.	%		
Diabetes mellitus						
Yes	24	38.7	40	64.5	8.267	0.003
No	38	61.3	22	35.5		
Hypertension						
Yes	8	12.9	42	67.7	38.742	0.000
No	54	87.1	20	32.3		

P<0.05:Significant.

4.5 Gastritis and peptic ulcer among the study population

The prevalence of gastritis and peptic ulcer among the study population is demonstrated in Table 4.5. Gastritis was reported in 17 (27.4%) cases compared to14 (22.6%) controls. The difference between the two group was not significant (χ^2=0.387, P=0.339). However, the reported peptic ulcer was significantly higher in cases compared to controls 10 (16.1%) vs. 3 (4.8%), $\chi^2_{(corrected)}$ =4.211 and P=0.038.

Table 4.5 Gastritis and peptic ulcer among the study population

Item	Controls (n=62)		Cases (n=62)		χ^2	p-value
	No.	%	No.	%		
Gastritis						
Yes	14	22.6	17	27.4	0.387	0.339
No	48	77.4	45	72.6		
Peptic ulcer						
Yes	3	4.8	10	16.1	4.211	0.038*
No	59	95.2	52	83.9		

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

4.6 Body mass index of the study population

Table 4.6 provides the BMI of the study population. The mean weight of controls was 82.5±9.4 Kg compared to 88.5±13.1 Kg of cases. The weight difference was significant (t=0.287 and P=0.004) with % difference=7.0%. There was a significant increase in the mean height of controls compared to cases (1.73±0.08 vs 1.68±0.07 m, % difference=2.9%, t=0.172 and P=0.000). Therefore, BMI was significantly increased in cases compared to controls (31.7±4.8 vs 27.6±4.4, % difference=13.8, t=0.864 and P=0.000).

Table 4.6 Body mass index of the study population

Anthropometric measurement	Controls (n=62) mean±SD	Cases (n=62) mean±SD	% difference	t	P-value
Weight (kg)* (min-max)	82.5±9.4 (62−100)	88.5±13.1 (58-122)	7.0	0.287	0.004
Height (m)** (min-max)	1.73±0.08 (1.58-1.90)	1.68±0.07 (1.55-1.82)	2.9	0.172	0.000
BMI*** (min-max)	27.6±4.4 (19.1-38.1)	31.7±4.7 (19.8-43.1)	13.8	0.864	0.000

*Kg: kilogram,** m: meter. ***BMI: Body mass index: Normal=18.5-24.9, Obeys ≥30 (WHO, 2012).All values are expressed as mean ±SD. P<0.05: Significant.

4.7 Cardiac enzyme activities of the study population

The mean activates of cardiac enzymes of the study population are presented in Table 4.7. There were significant elevations in the activities of AST and LDH in cases compared to controls (36.3±24.5 and 540.8±310.7 U/L vs 18.6±6.0 and 321.3±66.1 U/L, % difference=64.5 and 50.9, respectively [P=0.000]). Similarly CK and CK_{MB} activities were significantly higher in cases compared to controls (225.7±216.1 and 22.7±15.5 U/L vs 101.2±50.0 and 11.4±4.9 U/L, % difference=76.2 and 67.8, respectively [P=0.000]).

Table 4.7 Cardiac enzyme activities of the study population

Cardiac enzyme (U/L)	**Controls** (n=62) mean±SD	**Cases** (n=62) mean±SD	**% Difference**	**t**	**P-value**
AST (min-max)	18.6±6.0 (10-37)	36.3±24.5 (9-142)	64.5	5.533	0.000
LDH (min-max)	321.3±66.1 (235-502)	540.8±310.7 (255-1436)	50.9	5.440	0.000
CK (min-max)	101.2±50.0 (44-278)	225.7±216.1 (44-1103)	76.2	4.423	0.000
CKMB (min-max)	11.4±4.9 (4-31)	22.7±15.5 (6-93)	67.8	5.496	0.000

AST: Aspartate transaminase, LDH: Lactate dehydrogenate, CK: Creatine kinase, CKMB: Creatine kinase muscle brain all values are expressed as mean±SD. P<0.05: Significant.

4.8 Serum lipid profile of the study population

Table 4.8 illustrates serum lipid profile including cholesterol, triglycerides, HDL-C and LDL-C of the study population. The mean levels of cholesterol, triglycerides and LDL-C were significantly higher in cases (208.9±47.6, 218.0±110.1 and 131.6±41.9 mg/dl, respectively) compared to controls (174.8±34.1, 167.4±57.7 and 104.4±31.2 mg/dl, respectively) with % differences of 17.8%, 26.3%, and 23.1 and P=0.000, P=0.001 and P=0.000, respectively. On the other hand, the mean level of HDL-C was significantly lower in cases compared to controls (33.7±9.8 vs 37.6±8.4 mg/dl, % differences=10.9, P=0.020).

Table 4.8 Lipid profile of the study population

Lipid profile (mg/dl)	Controls (n=62) mean±SD	Cases (n=62) mean±SD	% difference	t	P-value
Cholesterol (min-max)	174.8±34.1 (116-266)	208.9±47.6 (114-333)	17.8	4.587	0.000
Triglycerides (min-max)	167.4±57.7 (63-323)	218.0±110.1 (31-600)	26.3	3.414	0.001
HDL-C (min-max)	37.6±8.4 (22-61)	33.7±9.8 (21-66)	10.9	2.355	0.020
LDL-C (min-max)	104.4±31.2 (59-188)	131.6±41.9 (56-242)	23.1	4.095	0.000

LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol. All values are expressed as mean ±SD. P<0.05: Significant.

4.9 Blood parameters of the study population

Table 4.9 illustrates blood parameters including white blood cell, red blood cell, hemoglobin and blood platelet of the study population. The white blood cell count was significantly higher in cases compared to controls (9.8±3.3 vs 7.8±1.6 $\times 10^9$/L, %difference 22.7, P=0.000). However, RBC count, hemoglobin content and platelet count did not show significant differences between cases and controls (P>0.05).

Table 4.9 Blood parameters of the study population

Blood parameters	**Controls** (n=62) mean±SD	**Cases** (n=62) mean±SD	**% Difference**	**t**	**P-value**
WBC×10^9/L (min –max)	7.8±1.6 (4.4-11.0)	9.8± 3.3 (4.8-19.1)	22.7	4.243	0.000
RBC×10^{12}/L (min –max)	4.6±0.55 (3.5-6.7)	4.5± 0.50 (3.4-5.9)	2.2	0.881	0.380
Hb (g/dl) (min –max)	12.7±1.8 (8.8-16.3)	13.0±1.6 (9.3-16.2)	2.3	1.061	0.291
PLT×10^9/L (min –max)	245.5±65.3 (147-500)	262.7± 83.9 (147-497)	6.8	1.271	0.206

WBC: White blood cell, RBC: Red blood cell, Hb: Hemoglobin, PLT: Platelet. All values are expected as mean ±SD. P>0.05: Not significant, P<0.05: Significant.

4.10 Distribution of *Helicobacter pylori* among the study population.

Distribution of *H. pylori* among the study population is presented in Table 4.10. 46 (74.2%) cases compared to 26 (41.9%) controls were positive for *H. pylori*. The difference between the two groups was significant (χ^2=13.248, P=0.000) with higher distribution of *H. pylori* among cases.

Table 4.10 Distribution of *Helicobacter pylori* among the study population

Family character	**Controls (n=62)**	**Cases (n=62)**	χ^2	**p-value**
	No. (%)	**No. (%)**		
***Helicobacter pylori* IgG**				
Positive	26 (41.9)	46 (74.2)	13.248	0.000
Negative	36 (58.1)	16 (25.8)		

P<0.05:Significant.

4.11 Relations of *Helicobacter pylori*

4.11.1 *Helicobacter pylori* infection in relation to gender among cases.

Table 4.11 shows the relationship between *H. pylori* and gender among cases. Although the number of males infected with *H. pylori* 25 (80.6%) was higher than that of females 21 (67.7%), the difference between the two groups was not significant (χ^2=1.384 and P=0.192).

Table 4.11 *Helicobacter pylori* infection in relation to gender among cases

Family character	Gender		χ^2	P-value
	Male (n=31)	Female (n=31)		
	n (%)	n (%)		
Helicobacter pylori				
Positive	25 (80.6)	21 (67.7)	1.384	0.192
Negative	6 (19.4)	10 (32.3)		

n: number of males and females, P>0.05:Not Significant.

4.11.2 *Helicobacter pylori* infection in relation to cardiac enzymes of cases

Table 4.12 gives the relationship between *H. pylori* and the cardiac enzymes. Although the activities of AST, LDH, CK and CK_{MB} enzymes were higher in *H. pylori* positive than negative cases, the differences between the two groups were not significant (P>0.05).

Table 4.12 *Helicobacter pylori* infection in relation to cardiac enzymes of cases

Cardiac enzymes (U/L)	*Helicobacter pylori*	n	mean±SD	t	P-value
AST	Positive Negative	46 16	42.1±34.7 34.4±19.8	1.088	0.281
LDH	Positive Negative	46 16	544.2±307.1 530.9±330.8	0.146	0.885
CK	Positive Negative	46 16	234.5±230.9 200.6±170.2	0.538	0.593
CK$_{MB}$	Positive Negative	46 16	23.5±13.0 22.4±16.4	0.235	0.815

AST: Aspartate transaminase, LDH: Lactate dehydrogenate, CK: Creatine kinase, CK$_{MB}$: Creatine kinase muscle brain. P>0.05: Not significant.

4.11.3 *Helicobacter pylori* infection in relation to lipid profile of cases

Helicobacter pylori in relation to cholesterol, triglycerides, HDL-C and LDL-C, are indicated in Table 4.13. The mean level of triglyceride in positive cases was significantly higher than that in negative cases (235.8±112.8 vs 166.6±85.7 mg/dl, P=0.029). The mean levels of cholesterol and LDL-C were also higher in positive than in negative cases (215.5±42.0 and 136.7±43.1 mg/dl, vs 189.7±35.8 and 116.9±35.7 mg/dl), but the differences between the two groups were not significant (P=0.061 and P=0.104, respectivily). On the other hand the mean level of HDL-C was significantly lower in positive compared to negative cases (31.7±8.0 vs 39.5±12.3 mg/dl, P=0.005).

Table 4.13 *Helicobacter pylori* infection in relation to lipid profile of cases

lipid profile (mg/dl)	*Helicobacter pylori*	n	mean±SD	t	P-value
Cholesterol	Positive Negative	46 16	215.5±42.0 189.7±35.8	1.912	0.061
Triglyceride	Positive Negative	46 16	235.8±112.8 166.6±85.7	2.234	0.029
HDL-C	Positive Negative	46 16	31.7 ±8.0 39.5±12.3	2.918	0.005
LDL-C	Positive Negative	46 16	136.7 ±43.1 116.9±35.7	1.652	0.104

HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol. n: number of positive and negative cases, P>0.05: Not significant, P<0.05: Significant.

4.11.4 *Helicobacter pylori* infection in relation to blood parameters of cases.

The relationship between *H. pylori* and blood parameters including WBC, RBC, Hb and PLT is presented in Table 4.14. The WBC count was significantly higher in positive compared to negative cases (10.5±3.5 vs 7.9±2.1 $\times 10^9$/L P=0.007). However, there were no significant differences in RBC, Hb and PLT of positive compared to negative cases (P>0.05).

Table 4.14 *Helicobacter pylori* infection in relation to blood parameters of cases

Parameter	*Helicobacter pylori*	n	mean±SD	t	P-value
WBC$\times 10^9$/L	Positive Negative	46 16	10.5±3.5 7.9±2.1	2.794	0.007
RBCs$\times 10^9$/L	Positive Negative	46 16	4.54±0.46 4.58± 0.59	0.316	0.753
Hb (g/dl)	Positive Negative	46 16	13.2±1.7 12.8±1.6	0.771	0.444
PLT$\times 10^9$/L	Positive Negative	46 16	269.1±89.6 244.1±63.9	0.308	0.308

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

4.11.5 *Helicobacter pylori* infection in relation to body mass index of cases

Table 4.15 shows the relationship between *H. pylori and the* body mass index of cases. There was no significant difference in body mass index between positive and negative cases (t=1.046, P=0.300).

Table 4.15. *Helicobacter pylori* in relation to body mass index of cases

Parameter	*Helicobacter pylori*	n	mean±SD	t	P-value
BMI (kg/m$^{3)}$	Positive Negative	46 16	32.7±4.2 30.7±4.2	1.046	0.300

BMI: Body mass index: Normal=18.5-24.9, Obeys ≥30 (WHO, 2012). P>0.05: Not Significant. N= number of positive cases and negative cases.

Chapter 5

Discussion

Cardiovascular disease remains the leading cause of death in the world as well as in the Palestinian territories. Approximately 80% of all cardiovascular-related deaths occur in low- and middle-income countries and at a younger age in comparison to high-income countries **(Gersh et al., 2010).** Despite that, the published data on the disease were limited in the Gaza strip and most of information emerged from annual reports produced by the Palestinian Ministry of Health. Recently two studies investigated the traditional risk factors associated with CAD in Gaza strip **(Khwaiter, 2009 and Mushtaha, 2010).** However, no previous study investigated *H. pylori* in CAD patients. Therefore, this is the first study to assess *H. pylori* infection as a risk factor for CAD in Gaza strip. Understanding the role of *H. pylori* in CAD could be useful in terms of prognosis and later management of the disease.

5.1 Socioeconomic data of the study population

The present study is a case control investigation included 62 CAD patients (31 males and 31 females) with mean age 53.1±7.3 years and 62 controls (31 males and 31 females) with mean age 52.8±7.0 years. The frequency of CAD was significantly higher among low educated individuals. This finding is in agreement with the study of **Vivek (2006)** who concluded that improving the level of education are likely to have a profound effect on the burden of premature CAD. In addition, CAD risk factors were reported to be associated with lower level of education in developing and developed countries **(Franks et al, 2011 and Abu Sedo, 2012).**

Data on employment showed a significant increase in CAD among unemployed individuals compared to employed ones. Similar result was obtained by **Naimi et al. (2009).** Concerning family income\month, CAD was more frequent among less income family. Such finding is in accordance with that found by **Franks et al.**

(2011) who stated that people with lower socioeconomic status are much more likely to developed heart disease than those who are wealthier. In addition, **Gersh et al. (2010)** reported that CAD remains the leading cause of death in the world and approximately 80% of all cardiovascular-related deaths occur in low- and middle-income countries in comparison to high-income countries.

Family history was found to be a risk factor of CAD. Literature review pointed out an association between family history and CAD **(Nasir et al., 2007; Sivapalaratnam et al., 2010 and Qureshi, 2012).** Regarding smoking, CVD was significantly more prevalent among smokers than non-smokers. It is known that smoking is a risk factor of CVD **(Hossain et al., 2007; de Bobadilla et al., 2010 and Arbab-Zadeh et al., 2012).** Tobacco smoke is both prothrombotic and atherogenic, increasing the risks of AMI, sudden cardiac death, stroke, aortic aneurysm and peripheral vascular disease. Even very low doses of exposure increase the risk of AMI **(Bullen, 2008).**

5.2 Physical activity and diet among the study population

Although the number of cases who doing exercise was significantly lower than controls, higher number of cases were found to follow diet. Physical inactivity was reported as a risk factor to be associated with the development of CAD. **(Kassam, and Stewart, 2001; Bassuk and Manson 2005; Weinstein et al.; 2008 and Lightfoot et al., 2010)**. In addition **Stewart (2013)** concluded that low physical activity was only partly explained by cardiovascular symptoms. The benefits of exercise can be explained on the basis of promoting weight reduction, helping reduce blood pressure, LDL-C and total cholesterol, and raising HDL-C levels **(Myers, 2003)**. The finding that higher number of cases followed diet implies that the CAD patients are aware of the role of diet in the management of the disease. In this context, **American Heart Association (2013)** reported that dietary habits affect multiple CVD risk factors, including both established risk factors (blood pressure, LDL-C levels, HDL-C levels, glucose levels, and obesity/weight gain) and novel risk factors (e.g. inflammation, cardiac arrhythmias and endothelial cell function).

5.3 Diabetes mellitus, hypertension and peptic ulcer among the study population

Diabetes mellitus and hypertension were more frequent among cases compared to controls. Such findings are in concurrent with results of other authors who reported that diabetes mellitus and hypertension are major contributors to CVD, and even directly or indirectly interfere and predict more serious complications of CAD **(Esteghamati et al., 2006; Oba et al., 2008; Dorjgochoo et al., 2009; Kathryn et al., 2010; Borg et al., 2011; Chiha et al., 2012 and Gulin, 2013)**. In addition, **Vats (2013)** was found that the risk for CAD is high among diabetic patients by the factor of 2 to 4 as compared to non-diabetics. Higher prevalence of diabetes mellitus and hypertension among CAD patients may be attributed to association of type 2 diabetes with endothelial dysfunction as it increases the inflammation and insulin resistance resulting in the increase of oxidized low density lipoprotein, endothelin 1, angiotensin II, oxidative stress and decreasing the action of nitric oxide and insulin or growth factors in endothelial cells **(Vats, 2013)**. In addition, **Dunlay (2010)** reported that hypertension and diabetes may lead to heart failure over longer durations via myocardial metabolic dysfunction, oxidative stress, and endothelial dysfunction, leading to left ventricular remodeling and cardiac dysfunction. The prevalence of peptic ulcer was significantly higher in cases than controls. In this context, **Tseng (2009)** concluded that peptic ulcer can occur with high prevalence in patients with CAD. In addition, the upper gastrointestinal bleeding was reported in CAD patients treated with low-dose aspirin **(William et al., 2006)**.

5.4 Body mass index of the study population

In the present study, BMI of cases was significantly higher than that of controls. In other words, obese individuals are at higher risk for CAD **(Tripta and Sharma 2013)**. The literature supported the present results in that obesity is a major risk factor of CAD **(Achari, 2006; Rocha and Libby 2009; Jonge et al., 2011 and Zahidullah et al., 2012)**. BMI as a measure of overall adiposity is strongly associated with increased prevalence of CAD independent of metabolic syndrome

(Yao et al. 2007). Chronic obesity leads to elevated macrophages, T cells, and numerous inflammatory mediators and pathways in the progression of atherosclerosis and obesity related metabolic disorders **(Rocha and Libby, 2009)**.

5.5 Serum lipid profile of the study population

The mean levels of cholesterol, triglycerides and LDL-C were found to be significantly increased in cases compared to controls. In contrast, HDL-C levels were significantly decreased in cases. Elevated levels of cholesterol, triglycerides and LDL-C, and low level of HDL-C were documented to be major risk factors for CAD (**Onat et al., 2006; Pollar, 2010; Miller et al., 2011 and Tomkin et al., 2012).** The previous findings that cases have higher BMI compared to controls and obesity contributed to CAD coincides with such alterations in lipid profile. High levels of cholesterol, triglycerides and LDL-C, and low level of HDL-C were recorded in obesity **(Bhatti et al., 2001; Thorpe et al., 2004 and Fox, 2011).**

5.6 Cardiac enzymes activities of the study population

Laboratory assay of cardiac enzymes showed significant elevations in the activities of AST, LDH CK and CK_{MB} in cases compared to controls. Similar results were previously reported **(Kemp et al., 2004 and Davey and Atlee, 2011).** The increments in the activities of these enzymes are expected as they believed to be specific biomarkers of CVD. Despite multiple known associations of elevated serum cardiac markers in patients with congestive heart failure, the presence of clinical presentation of ACS and elevated markers should alert the practitioner to the potential for identifiable coronary artery pathology **(Glauser et al. 2007).**

5.7 Hematological profile of the study population

White blood cell were significantly increased in cases compared to controls whereas no significant changes were found in RBC count, hemoglobin content and

platelet count between cases and controls. Leukocytosis was reported in CAD patients **(Libby et al., 2010 and Moore et al., 2011)**. The reason of Leuckocytosis in CVD is still not fully understood. The proposed mechanism is based on the idea that elevation of WBC count may enhance atherogenesis and is also reflected in the inflammatory activity of atherosclerosis that causes vascular injury and tissue ischemia **(Stocker et al., 2004 and Wu et al., 2013).** In addition, WBC count admitted as an independent risk factor for CAD or even as independent marker of cardiac mortality (**Sulaiman et al., 2012; Bhat et al., 2013 and Salehi et al., 2013).**

5.8 Distribution of *Helicobacter pylori* among the study population

The results of this study showed significantly higher positive *H. pylori* infection 46 (74.2%) among CAD patients compared to controls 26 (41.9%). This marked increment in *H. pylori* infection in CAD patients indicates that *H. pylori* is associated with CAD. Higher prevalence of *H. pylori* was found among CAD patients **(Küçükardal, 2009**; **AL-Obeidy and Saeedet, 2011; Vareki et al., 2013 and Viswanath et al., 2013)**. The *H. pylori* infection in relation to other studied parameters amonge cases was assecd. When related to *H. Pylori*, triglyceride levels were significantly higher in positive than that in negative cases, whereas HDL-C levels were significantly lower in positive cases. Similar results were previously reported (**Abu Al- Soud et al., 2008 and Viswanath et al., 2013)**. The mechanism of how *H. pylori* infection modifies the serum lipid profiles is still not clear, but a plausible explanation is that systemic inflammatory response to the bacterium induces changes in lipid and lipoprotein metabolism **(Khovidhunkit et al., 2000)**. That is, chronic *H. pylori* infection has been postulated to shift the lipid profile toward an atherogenic direction via the action of proinflammatory cytokines, such as interleukins 1 and 6, interferon-alpha, and tumor necrosis factor alpha (TNF-α). These cytokines are capable of affecting lipid metabolism in various ways, including activation of adipose tissue lipoprotein lipase, stimulation of hepatic fatty acid synthesis, influencing lipolysis and the increasing hepatic

Hydroxyl methy glutary-CoA (HMG-CoA) reductase activity **(Khovidhunkit et al., 2004)**. Thus, *H. pylori* infection could play a role in the atherosclerotic process and may be a reliable indicator for the assessment of CVD risk **(Lim et al., 2013)**. This is supported the self-reported complication of CVD reported in the present study. When related to *H. pylori*, the WBC count was significantly higher in positive compared to negative cases. Leukocytosis was reported in *H. pylori* infected patients **(Koenig et al., 1999 and Al Soud et al., 2008)**. 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 **(Zade et al, 2009 and Iida et al., 2012)**.

Chapter 6

Conclusions & Recommendations

6.1 Conclusions

1. Coronary artery disease was more prevalent among less educated and unemployed individuals, families with low income, individuals with family history of the disease as well as among smokers.

2. The number of cases doing exercise was significantly lower than controls. However, the number of cases who followed diet was significantly higher than controls.

3. The number of cases who had diabetes mellitus, hypertension and peptic ulcer was significantly higher than that of controls.

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

5. The activities of cardiac enzymes AST, LDH, CK and CK_{MB} were significantly elevated in cases compared to controls.

6. The levels of cholesterol, triglycerides and LDL-C were significantly higher in cases compared to controls, whereas the level of HDL-C was significantly lower in cases.

7. The white blood cell count was significantly higher in cases compared to controls.

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

10. When related to *H. pylori*, serum triglycerides levels were significantly increased in *H. pylori* positive cases than in negative cases, whereas HDL-C level was significantly lower in positive cases.

11. The WBC count was singnificantly elvated in *H. pylori* positive cases compared to negative cases.

6.2 Recommendations

1. Frequent monitoring of *H. pylori* infection as a risk factor of coronary artery disease.

2. Estimation of lipid profile and WBC count is recommended to avoid the deleterious effect of *H. pylori* infection associated with CAD.

3. Quitting smoking and doing exercising are recommended to decrease the risk of CAD, particularly in persons with family history of the disease.

4. Further research is highly recommended on the role of *H. pylori* in pathogenesis mechanism of CAD and *H. pylori* infection among other chronic diseases.

References

Abu Al-Soud A., Mostafa A., El-Sayed S. and Mahmoud A. (2008): The Role of *Helicobacter pylori* Infection in Patients with Chest Pain. Menoufiya Medical Journal Vol.21 No.1

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. MSC thesis, El Azhar University of Gaza, Palestine

Abu Sedo R. R. (2012): Homocysteine levels of cardiovascular disease patients attending the cardiac unit at El Shifa hospital, Gaza Strip. MSC thesis, IUG of Gaza, Palestine

Abu-Mughesieb R. (2007): Risk Factors Associated with *Helicobacter pylori* Infection in Gaza, Palestine. MSC thesis, IUG of Gaza, Palestine

Achari V., Thakur A. K. and Sinha A. K. (2006): The Metabolic Syndrome – Its Prevalence and Association with Coronary Artery Disease in Type 2 Diabetes. JIACM 2006; 7(1): 32-8

Anderson J.L., Adams C.D., Antman E.M., Bridges C.R., Califf R.M., Casey D.E. and Chavey W.E. (2007): Guidelines for the management of patients with unstable angina/non–ST-elevation myocardial infarction. Journal of American College of Cardiology, 50: 150-157.

Annuziata P, Figura N, Galli R, Murganini F, Lenzi C., (2003): Association of anti-GM1 antibodies but not of anti-cytomegalovirus, *campylobacter jejuni* and *Helicobacter pylori* IgG, with a poor outcome in Guillain-Barre syndrome. J Neurol Sci; 213: 55-60.

Arbab-Zadeh Armin., Masataka Nakano., Renu Virmani., and Valentin Fuster. (2012): Acute coronary. Circulation, 125: 1147-1156

Asfeldt A.M., Steigen S.E., Løchen, M.L., Straume B., Johnsen R., Bernersen B., Florholmen J., and Paulssen E.J. (2009): The natural course of *Helicobacter pylori* infection on endoscopic findings in a population during 17 years of follow-up: the Sørreisa gastrointestinal disorder study. European Journal of Epidemiology. 24(10): 649-658.

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

Assareh A. R., Cheraghi M., Nourizadeh M., Daeenejad F., Haybar H, Kiarsi M. R. (2013): Distributions of ischemic heart disease risk factors in patients who were admitted for angioplasty in Iran. World Journal of Cardiovascular Diseases 3, 45-49.

Azarkar Z., Jafarnejad M., Gholamreza and Sharifzadeh (2011): The relationship between *Helicobacter pylori* infection and myocardial infarction. Caspian J Intern Med; 2 (2):222-225.

Backestrom, C. and Hursh-Cesar, G. (2012): Survey Research, Pennsylvania, United States: Literary Licensing, LLC.

Bassuk, S., and Manson, J., (2005): Epidemiological evidence for the role of physical activity in reducing risk of type 2 diabetes and cardiovascular disease. Journal of Applied Physiology, Sep., 99 (3): 1193-1204.

Bender, 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. Turk J Gastroenterol. 18:225-9.

Bernard G., Sliwa K., Mayosi B. M. and Yusuf S. (2010): The epidemic of cardiovascular disease in the developing world, global implications European Heart Journal Advance.

Bhat T., Teli S., Rijal J., Bhat H., Raza M., Khoueiry G., Meghani M., Akhtar M. and Costantino T. (2013): Neutrophil to lymphocyte ratio and cardiovascular diseases. Expert Review of Cardiovascular Therapy Vol. 11, No. 1, Pages 55-59,

Bhatti M., Akbri M.Z., and Shakoor M. (2001): Lipid profile in obesity. Journal of Ayub Medical College, Abbottabad, 13(1): 31-33.

Borg R., J. C. Kuenen., B. Carstensen., H. Zheng., D. M. Nathan., R. J. Heine., J. Nerup K. Borch-Johnsen and D. R. Witte. (2010): HbA1c and mean blood glucose show stronger associations with cardiovascular disease risk factors than do postprandial glycaemia or glucose variability in persons with diabetes, The A1C-Derived Average Glucose (ADAG) study. Diabetologia 54: 69–72.

Borg R., Kuenen J. C., Carstensen B., Zheng H., Nathan D. M., Heine R. J., Nerup J., Borch-Johnsen K. and Witte D. R. (2010): HbA1c and mean blood glucose show stronger associations with cardiovascular disease risk factors than do postprandial glycaemia or glucose variability in persons with diabetes, The A1C-Derived Average Glucose (ADAG) study. Diabetologia 54: 69–72.

Braekkan S. K., Mathiesen E. B., Njølstad, Wilsgaard T., Størmer J. and Hansen J. B. (2008): Family history of myocardial infarction is an independent risk factor for venous thromboembolism. The Tromsø study Journal of Thrombosis and Haemostasis. 6: 1851–1857.

British Heart Foundation (2008): European Cardiovascular Disease Statistics. International cardiovascular disease statistics, Statistical fact sheet populations, http://www.americanheart.org/downloadable/heart/L.pdf. (Accessed on Nov. 2013)

Brown, L.M. (2000): *Helicobacter pylori*: epidemiology and routes of transmission. Epidemiol Rev. 22(2):283-97.

Bucolo G and David H. (1973): Quantitative determination of serum triglycerides by use of enzymes c/in chern. 19: 476-482.

Bullen Christopher. (2008): impact of tobaco smoking and smoking cessation on cardiovascular risk and disease. Expert Rev.Cardiovas.Ther.6 (6): 883-895.

Cecie Starr; Christine Evers; Lisa Starr (2009): Biology: Today and Tomorrow With Physiology. Cengage Learning, p. 422.

Centers for Disease Control and Prevention CDC, (2009): Heart Disease. Coronary Artery Disease),
http://www.cdc.gov/heartdisease/coronary_ad.htm. Accessed Aug, 2013

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.

Chiha M., Njeim M., and Chedrawy E. G. (2012): Diabetes and Coronary Heart Disease: A Risk Factor for the Global Epidemic. International Journal of Hypertension Volume 2012, Article ID 697240, page 7

Christian Weber & Heidi Noels (2011): Atherosclerosis: current pathogenesis and therapeutic options. Nature medicine volume 17, number 11

Collazo, S. (2012): *Helicobacter pylori*: Toward effective eradication Issue of Clinical Advisor March 2012.

Cunningham Joan. (2010): Socioeconomic disparities in self-reported cardiovascular disease for Indigenous and non-Indigenous Australian adults, analysis of national survey data, Cunningham Population Health Metrics. 8: (31).

Danesh J, Collins R, Peto R., (1997): Chronic infections and coronary heart disease: is there a link? Lancet; 350:430–6.

Davey MS. and Atlee CW, (2011): Inotropic and cardio protective effect of terminalla paniculata roth bark extract in doxorubicin induced cardio toxicity in rats. International journal of research jn Ayurveda and pharmacy, 2(3): 869-875.

De Bobadilla J. F., Burgoab V. S., Morales P. Garrido E., Sád L. (2011): Riesgo cardiovascular: evaluación del tabaquismo y revisión en atención primaria del tratamiento y orientación sanitaria. Estudio Retratos 10: 1016.

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 Pract Res Clin Gastroenterol. 21 (2): 215–36.

Demosthenes B., Dean K., Alessandro M. and Chrysohoou C. (2005): The relation between pulse pressure and cardiovascular mortality in 12763 middle-aged men from various parts of the world, Journal of American Medical Association. 165: 2142-2147.

Di angilantonio E., Sarwar N., Kaptoge P. and Ray K. (2009): Major lipids, apolipoproteins, and risk of vascular disease, Journal of the American Medical Association. 302: 1993-2000.

Dorigochoo T., Shu X. and Zhang X. (2009): Relation of blood pressure components and categories and all-cause, stroke and coronary heart disease mortality in Urban Chinese women, A population-based prospective study. Journal of Hypertension, 27: 468-475.

Dunlay S. M., Weston S. A., Jacobsen S. J. and Roger V. L. (2010): Risk Factors for Heart Failure: A Population-Based Case-Control Study. Am J Med. Author manuscript; available in PMC.

EMedicine - Coronary Artery Atherosclerosis Article by Singh V. N.mht. (2013): http://www.emedicine.com/med/byname/Coronary-Artery-Atherosclerosis.htm. (Accessed on Nov. 2013)

EMedicine - Myocardial Ischemia Article by Zevitz M. E.mht, (2013): http://www.emedicine.com/med/byname/Myocardial-Ischemia.htm. (Accessed on Nov. 2013)

EMedicine - Unstable Angina Article by Walter A T.mht, (2013): http://www.emedicine.com/med/byname/unstable-angina.htm. (Accessed on Nov. 2013)

Encyclopedia of science (2013): Heart topics, coronary artery. (Accessed on Nov. 2013)

Enroth H. and Wreiber 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 mother child transmission of *Helicobacter pylori* infection. Arq Gastroenterol, 41: 239–244.

Esteghamati A., Abbasi M. and Nakhjavani M. (2006): Prevalence of diabetes and other cardiovascular risk factors in an Iranian population with acute coronary Syndrome. Cardiovascular Dialectology 5-15.

Esteghamati A., Abbasi M. and Nakhjavani M. (2006): Prevalence of diabetes and other cardiovascular risk factors in an Iranian population with acute coronary Syndrome. Cardiovascular Dialectology 5-15.

Fox S. (2011): High Triglycerides in Obesity: Dual Metabolic Defects, Arteriosclerosis, Thrombosis and Vascular Biology. Journal of the American Heart Association

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

Franks P., Winters C. P., Tancredi J. D. and Fiscella A. K. (2011): Do changes in traditional coronary heart disease risk factors over time explain the association between socio-economic status and coronary heart disease? BMC Cardiovascular Disorders (7): 1-7.

Fransson E., Faire U. and Ahlbom A. (2006): The effect of leisure-time physical activity on the risk acute myocardial infraction depending on body mass index, a population-based case-control study. Biomed central Public Health, 6: 290-296.

Fretts, A.M. Howard, B.V. Kriska, A.M. Smith, N.L. Lumley, T. and Lee, E.T. (2009): Physical activity and incident diabetes in American Indians. The strong Heart Study. A J Epidemiology 170(5):632-9.

Friedewald W.T., Levy R.I. and Fredrickson D.S. (1972): Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry 18 (6): 499-502.

German Society for Clinical Chemistry (1977): Recommendations of the Enzyme Commision. J. Clin. Chem. Clin. Biochem 15: 255.

German Society for Clinical Chemistry, Recommendations of the German Society for Clinical Chemistry (DGKC) (1972): Standardization of methods for the determination of enzyme activities in biological fluids (Recommendations of the German Society of Clinical Chemistry Standardization of methods for measurement of enzymatic activities in biological fluids.) Z Klin Chem Klin Biochem, 10: 182-92.

Gersh j. B., Karen S., Bongani M. M. and Salim Y. (2010): The epidemic of cardiovascular disease in the developing world, global implications European Heart Journal Advance Access published February 22.

Glauser J., Erickson J., Bhatt D., Lindsell C., Gibler B,, Hoekstra J., Pollack C., Hollander J., Peacock W. F. (2007): Elevated Serum Cardiac Markers Predict Coronary Artery Disease in Patients With a History of Heart Failure Who Present With Chest Pain: Insights From the itrACS Registry. Congestive Heart FailureVolume 13, Issue 3, page 142-148.

Goodwin, C.S & Worsley, B.W. (1993): Microbiology of *Helicobacter pylori*. Gastroenterol Clin North Am. 22: 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. nov*., Respectively, Int J Syst Bacteriol. 39: 397–405.

Grove TH. (1979): Effect of reagent pH on determination of HDL-C by precipitation with sodium phosphotungastate-magnesium. Clin Chern. 255-260

Gulin D., Galić E., Vrbanić L., Kordić K., Šikić J. (2013): Distribution of coronary artery disease in acute coronary syndrome patients with diabetes mellitus. Journal of Cardiothoracic Surgery, 8 P151

Gupta R., Gupta V.P., Sarna M., Prakash H., Rastogi S. and Gupta K.D. (2003): Serial epidemiological surveys in an urban Indian population demonstrate increasing coronary risk factors among the lower socioeconomic strata. J Assoc Physicians India 51: 470-7.

Haldeman G.A., Croft J.B., Giles W.H. and Rashidee (1999): Hospitalization of patients with heart failure, National Hospital Discharge Survey, 1985 to 1995. Am Heart J. 137: 352-360.

Hansson G. K.: (2005): Mechanisms of disease Inflammation, Atherosclerosis, and Coronary Artery Disease. N engl j med 352; 16

Hasanaj Q.B., Wilson J., Little J., Montazeri J.C., (2013): Family History: Impact on Coronary Heart Disease Risk Assessment beyond Guideline-Defined Factors. Public Health Genomics 16(5):208-14.

Hayes D.K., Greenland K.J., Denny C.H., Keenan N.L. and Croft J.B. (2005): Disparities in multiple risk factors for heart disease and stroke, MMWR. 54: 113-116.

Hazell, S.L, Lee, A. Brady, L. Hennessy, W. (1986): *Campylobacter pylori* is and gastritis: association with intercellular spaces and adaptation to an environment of mucus as important factors in colonization of the gastric epithelium. J Infect Dis. 153: 658–663.

Heart encyclopedia web site (1990): Biostatistical fact sheet-populations, http://www.ipc-india.com/heart-encyclopedia/page/23.asp (accessed on 9/2013).

Hossain P., Kawar B. and El Nahas M. (2007): Obesity and Diabetes in the Developing World — A Growing Challenge, The New England Journal of Medicine. 356 (3): 213-215.

Hossain P., Kawar B. and El Nahas M. (2007): Obesity and Diabetes in the Developing World — A Growing Challenge, The New England Journal of Medicine. 356 (3): 213-215. http://www.cdc.gov/heartdisease/coronary_ad.htm. (Accessed on Nov. 2013)

Hu, F.B. Li, T.Y. Colditz, G.A. Willett, W.C. and Manson J.E. (2003): Television watching and other sedentary behaviors in relation to risk of obesity and type 2 diabetes mellitus in women. JAMA 289(14):1785-91.

Iida M., Ikeda M., Ninomiya F., Yonemoto T., Doi K., Hata Y., Matsumoto J., Iida T., 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

Karlamangla A.S., Stein Merkin S., Crimmins E.M. and Seeman T.E. (2010): Socioeconomic and ethnic disparities in cardiovascular risk in the United States, 2001-2006. Ann Epidemiol. 20: 617-628.

Kassam S., And Stewart D., (2001): Novel risk factors for coronary artery disease. Cardiology, v o l u m e VI, i s s u e 8 page 102-107

Kate, V. Maroju, N.K. and Ananthakrishnan, N. (2013): *Helicobacter pylori* Infection and Upper Gastrointestinal Disorders. Gastroenterology Research and Practice, Hindawi Article ID 896209, 3 pages

Kelalainen P., Sarlund H., Pyorala K. and Laakso M. (1996): Family history of coronary heart disease is a strong predictor of coronary heart morbidity and mortality than family history of non-insulin dependent diabetes mellitus, Atherosclerosis. 123: 203-213.

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

Khovidhunkit W., Memon, R.A. Feingold K.R. and Grunfeld, C. (2000): Infection and Inflammation-Induced Proatherogenic Changes of Lipoproteins. J Infect Dis. 181(3): 462-472.

Khovidhunkit, W. Kim, M.S. Memon, R.A. Shigenaga, J.K. Moser, A.H. Feingold, K.R. and Grunfeld, C. (2004): The Pathogenesis of Atherosclerosis. Effects of infection and inflammation on lipid and lipoprotein, 45(7):1169-96. Epub

Khwaiter, S.H. (2009): Risk Factors Associated with Coronary Artery Disease in Gaza. MSC thesis, IUG of Gaza, Palestine

Koenig W., Rothenbacher D., Hoffmeister A., Miller M., Bode G., Adler G., Hombach V., Ma¨rz W., Pepys M. B., Brenner H. (1999): Infection With

Helicobacter pylori Is Not a Major Independent Risk Factor for Stable Coronary Heart Disease Lack of a Role of Cytotoxin-Associated Protein A–Positive Strains and Absence of a Systemic Inflammatory Response. Circulation; 100:2326-2331

Kugiyama K., Doi H., Takazone K. and Kawano H. (1999): Remnant lipoprotein levels in fasting serum predict coronary events in patients with coronary artery disease, Circulation. 99: 2858-2860.

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

Lane, J.A. Murray, L.J. Sian, N. Egger, M. Harvey, I.M. and Donovan, J.L. (2006): Impact of Helicobacter pylori eradication on dyspepsia, health resource use, and quality of life in the Bristol helicobacter project: randomized controlled trial. BMJ 332(7535): 199–204.

Lavie J. C., Richard V. M., Hector O. V, and Louisiana (2009): Obesity and Cardiovascular Disease Risk Factor, Paradox, and Impact of Weight. Loss Journal of the American College of Cardiology, 53 (21)

Layla L D. J., Osch-Gevers A., Willemsen S., Steegers E., Hofman A., Helbing W. and Jaddoe V. (2011): Growth, Obesity, and Cardiac Structures in Early Childhood, Erasmus Medical Center, Rotterdam, The Netherlands.

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

Lehto S., Ronnemaa T., Haffner S., Pyorala K., Kallio V. and Laakso M. (1997): Dyslipidemia and hyperglycemia predict coronary heart disease events in middle-aged patients with NIDDM, Diabetes. 46: 1354-1359.

Lembo L., Caradonna T., Magrone M. L., Mastronardi D., Caccavo E., Jirillo L., Amati (2005): Immunobiology: The Immune System in Health and Disease 6th Ed. Charles A. Janeway Jr. et al. .

Libby P., Okamoto Y., Rocha V. Z., (2010): Inflammation in Atherosclerosis: Transition From Theory to Practice. Circ J 2010; 74: 213 – 220)

Lightfoot Kathryn Ann. (2010): Body image and physical activity in people living with heart disease, MSC thesis Dalhousie University Halifax, Nova Scotia.

Lim S.H., Kwon J.W., Kim N., Kim G.H., Kang J.M., Park M.J., Yim J.Y., Kim H.U., Baik G.H., Seo G.S., Shin J.E., Joo Y.E., Kim O.S. and Jung H.C. (2013): Prevalence and risk factors of *Helicobacter pylori i*nfection in Korea: Nationwide multicenter study over 13 years. BMC Gastroenterology 13(104): 1-10.

Mähönen M.S., McElduff P., Dobson A.J., Kulasmaa K.A. and Evans A.E. (2004): Current smoking and risk of non-fatal myocardial infraction in the WHO MONICA project populations, Tobacco control. 13: 244-250.

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

Mannan H. R., Stevenson C. E., Peeters A., He Walls l. L. and McNeil J. J. (2011): Age at quitting smoking as a predictor of risk of cardiovascular disease incidence independent of smoking status, time since quitting and pack-years, Biomed Center Research.1-9.

Marieb E.M. (2011): Essentials of Human Anatomy & Physiology, 10th Edition, Amazon.com.

Marković B. B., Vrdoljak D., Kranjčević K., Vučak J., Kern J., Bielen I., Ivezić Lalić D., Katić . and Reiner Z., (2011): Continental-Mediterranean and rural-urban

differences in cardiovascular risk factors in Croatian population. Crroatian medical jornal 52(4): 566–575.

Markus HS, Mendall MA., (1998): *Helicobacter pylori* infection: a risk factor for ischaemic cerebrovascular disease and carotid atheroma. J Neurol Neurosurg Psychiatry; 64:104–7.

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

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.

Meiatlini F., prencipe L., Bardelli F.,Giannini G. and Tarli P. (1978): The zone 4-hydroxybenzoate/4aminophenazone chromogenic system used in the enzymic determination of serum cholesterol. Clin Chem, 24: 2161-2165.

Meinrad G., (2004): Role of platelets in coronary thrombosis and reperfusion of ischemic myocardium. Cardiovascular Research 61: 498. 51.

Michael M., Stone N., Christie B., Vera B., Michael C., Henry G., Carol G. A., James H. W., Marc J., Penny K., Terry L., Moshe L., Theodore M. and Subramanian P. (2011): Triglycerides and Cardiovascular Disease. A Scientific Statement from the American Heart Association, Circulation, 123:2292-2333.

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. J Infect Dev Ctries. 1; 2(3):206-10.

Miyazaki M., Babazono A., Kadowaki K., Kato M., Takata T., Une H., (2006): Is *Helicobacter pylori* infection a risk factor for acute coronary syndromes? Journal of Infection 52, 86–91.

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 J Gastroenterol. 18(17): 2105–2111.

Moore K. J. and Tabas I., (2011): Macrophages in the Pathogenesis of Atherosclerosis. Cell 145, page 341-355

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

Mushtaha, M.N. (2010): Risk factors of in Coronary Artery Disease patients undergoing cardiac catheterization in Gaza governorates: Case-control study.

Naimi A. I., Paquet C., Lise G. and Daniel M. (2009): Associations between Area-Level Unemployment, Body Mass Index, and Risk Factors for Cardiovascular Disease in an Urban Area. Int. J. Environ. Res. Public Health 6(12)

Nakamura K., Toru M., Kenki E., Motoki ., Masato M., Yoshiki H., Kunihisa K., Hiroshi M., Kengo F. K. And Hiroshi I. (2011): Impact of Hypertriglyceridemia on Endothelial Dysfunction During Statin ± Ezetimibe Therapy in Patients With Coronary Heart Disease, American Journal of Cardiology 108 (3): 333-339.

Nasir K., Donnelly E. and John A.R. (2004): Coronary artery calcification and family history of premature coronary heart disease, Circulation. 110: 2150-2156.

Nasir K., Matthew J., Wong N., Scheuner M. and Herrington D. (2007): Family history premature coronary heart disease and coronary artery calcification, Circulation. 116: 619-626.

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.

Nozari Y., Akiash N., Daryani N. E., Abdollahi A., (2009): Association between *Helicobacter pylori* Infection and Atherosclerotic Coronary Artery Disease. Iranian Journal of Pathology, 4 (1), 1- 4.

Nurgalieva, Z.Z. Malaty, H.M. Graham, D.Y. Almuchambetova, R. Machmudovaet, A. and Kapsultanova, D. (2002): *Helicobacter pylori* infection in Kazakistan: effect of water source and household hygiene. Am J Trop Med Hyg. 67: 201-206.

Oba S., Nagata C., Nakamura K. and Shimizu H. (2008): Self-reported diabetes mellitus and risk of mortality from all causes, cardiovascular disease, and cancer in Takayama, A population-based prospective cohort study in Japan. Epidemiology Journal, 18: 197-203.

Oladapo O. O., Salako L., Sadiq L., Soyinka K. and Falase A. O. (2013): Knowledge of Hypertension and other Risk Factors for Heart Disease among Yoruba Rural Southwestern Nigerian Population. British Journal of Medicine & Medical Research 3(4): 993-1003.

Onat A, Sarı I, Yazıcı M, Can G, Hergenç G. and Avcı G.S. (2006): Plasma triglycerides, an independent predictor of cardiovascular disease in men: A prospective study based on a population with prevalent metabolic syndrome. International Journal of Cardiology 108 (1): 89-95.

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 profoundsocioeconomic changes. Helicobacter, 9(3):233–241.

Park M. J., Cho S. H., Kim D, Kang S. J., Chung S. J., Choi S. Y., Yoon D. H., Lim S. H., Kim Y. S., Yim J. Y., Kim J. S., and Jung H.C. (2011). Association between *Helicobacter pylori* Seropositivity and the Coronary Artery Calcium Score in a Screening Population, Gut Liver; 5(3): 321–327.

Pischon T., Girman C., Sacks F., Rifai N., Stampfer M. and Rimm E. (2005): Non-high density lipoprotein cholesterol and apo lipoprotein B in the prediction of coronary heart disease in men, Circulation. 112: 3375-3383.

Pollar.janet. (2010): Heart disease risk factors, screenings, changes.health hints 14(2).

Qureshi N., Armstrong S., Dhiman P., Saukko P., Middlemass J. and Evans PHand Kai J. (2012): Effect of Adding Systematic Family History Enquiry to Cardiovascular Disease Risk Assessment in Primary Care: A Matched-Pair. Cluster Randomized Trial. 156 (4): 253-262.

Reddy K., Prabhakaran D., Jeemon P., Thankappan K. and Joshi P. (2007): Educational status and cardiovascular risk profile in Indians, PNAS. 104: 16263-16268.

Rocha V. Z. and Libby P. (2009): Obesity, inflammation, and atherosclerosis. Cardiology 6, 399-409

Saadalah N. (2013): Assessment of the *H. pylori* infection as a risk factor for type 2 diabetes mellitus in Gaza strip. MSC thesis, IUG of Gaza, Palestine

Sachs, G. and Scott, R.D. (2012): Helicobacter pylori: Eradication or Preservation. F1000 Med. Rep.4-7

Sadeghi R., Adnani N., Erfanifar A., Gachkar L., Maghsoomi Z. (2013): Premature Coronary Heart Disease and Traditional Risk Factors-Can We Do Better? Int Cardiovasc Res; 7(2):46-50.icrj.11408

Sakakura K., Nakano M., Otsuka F., Ladich E., Kolodgie F., Virmani R. (2013): Pathophysiology of Atherosclerosis Plaque Progression. Heart, Lung and Circulation Volume 22, Issue 6, Pages 399–411

Salehi N, Eskandarian R, Sanati H. R., Firouzi A., Shakerian F., Abdi S., Bakhshandeh H., AhmadAbadi M. N., Nouri N., Vakili-Zarch A. (2013): White blood cell count and mortality in acute myocardial infarction. World Journal of Cardiovascular Diseases, 2013, 3, 458-463.

Sawayamaa Y., Ariyamaa I., Hamadaa M., Otaguroa S., Machib T., Tairac Y., Hayashid J., (2005): Association between chronic *Helicobacter pylori* infection and acute ischemic stroke: Fukuoka Harasanshin Atherosclerosis Trial (FHAT). Atherosclerosis 178 303–309.

Sebastián F. A., Esteban A. F., Ramón C. L., Gustavo E. S. (2001): Detection of *Helicobacter pylori* in Human Carotid Atherosclerotic Plaques. Stroke.; 32:385-391

Sesso H.D., Lee I.M. and Gaziano J.M. (2001): Maternal and paternal history of myocardial infraction and risk of cardiovascular disease in men and women, Circulation. 104: 393-398.

Shiota S., Murakawi K., and Yamaoka Y. (2013): *Helicobacter pylori* infection in Japan. Expert Review of Gastroenterology & Hepatology, 7(1), P35-40

Shiraishi J., Kohno Y., Sawada T. and Nishizawa S. (2006): Relation of obesity to acute myocardial infraction in Japanese patients-differences in gender and age, Circulation. 70: 1525-1530.

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. Arch Oral Biol. 54(7):684-8.

Sivapalaratnam S., Boekholdt S.M., and Trip M.D. (2010): Family history of premature coronary heart disease and risk prediction in the EPIC Norfolk prospective population study. Heart 96:1985-1989.

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. Invest Clin. 47(2): 109-116.

Stasi, R. and Provan, D. (2008): *Helicobacter pylori* and Chronic ITP. Hematology Am Soc Hematol Educ Program, 206-211.

Stenström B., Mendis A., Marshall B., (2008): "*Helicobacter pylori* - The latest in diagnosis and treatment". Aust Fam Physician. 37 (8): 608–12.

Stewart R., Held C., Brown R., Vedin O., Hagstrom E., Lonn E., Armstrong P., Granger C. B., Hochman J., Davies R., Soffer J., Wallentin L., and Lane H., (2013): Physical activity in patients with stable coronary heart disease: an international perspective. European Heart Journal

Stocker R, John F, Keaney J., (2004): Role of Oxidative Modifications in Atherosclerosis, Physiol. Rev. 84: 1381-1478.

Suerbaum S, and Michetti P. (2002): *Helicobacter pylori* infection. N Engl J Med 347:1175-86.

Sulaiman Ad., Fesc F., Al-Zakwani I., Panduranga P., Al-Suwaidi J., Alsheikh A. A., Al Mahmeed W., Amin H., Al Mutarreb A., AlHabib K., Al Lawati J., and

Zubaid M. (2012): Relationship between White Blood Cell Count and In-Hospital Outcomes in Acute Coronary Syndrome Patients from the Middle East. Angiology 63:24.

Tewari R., Nijhawa V.S., Mishr M.N., Dudeja P., Salopal TK. (2012): Prevalence of H*elicobacter pylori, cytomegalovirus,* and *Chlamydia pneumoniae*

immunoglobulin seropositivity in coronary artery disease patients and normal individuals in North Indian population. Medical Journal Armed force India, Volume 68, Issue 1 , Pages 53-57.

Thomas A. Gaziano. (2005): Cardiovascular Disease in the Developing World and Its Cost-Effective Management, Circulation; 112:3547-3553.

Thomas L. (1998): Alanine aminotransferase (ALT), Aspartate aminotransferase (AST). Clinical Laboratory Diagnostics, 1st ed, Frankfurt, TH-Books Verlagsgesellschaft. p. 55-65.

Thorpe K.E., Florence C.S., Howard D.H. and Joski P. (2004): The impact of obesity on rising medical spending, Health Affairs: W4-480-6.

Tigran K, Davtyana b, Hayk A, Manukyana R, Mkrtchyanc A, Samvel A, Armen A., (2005): Galoyan Hypothalamic Proline-Rich Polypeptide Is a Regulator of Oxidative Burst in Human Neutrophils and Monocytes. Neuroimmunomodulation; 12:270-284.

Tiwari S.K., Khan A.A., Ahmed K.S., 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 Med 46:224-8.

Tomkin G. and Owens D. (2012): LDL as a Cause of Atherosclerosis. The Open Atherosclerosis & Thrombosis Journal 5, 13-21.

T**ripta and Sharma K., (2013):** Body Mass Index, Blood Lipid and Apolipoprotein levels and Coronary Heart Disease among middle aged Punjabi Khatris of Northwest India. Human Biology Review 2 (4)

Tsai WC, Li YH, Sheu BS, et al., (2001): Association of elevation of anti-*Helicobacter pylori* antibody with myocardial ischemic events in coronary artery disease. AM J Cardiol; 87: 1005-7.

Tseng P.H., Liou J.M., Lee Y.C., Lin L.Y., Yan-Zhen L. A., Chang D.C., Chiu H.M., Wu M.S., Lin J.T., Wang H.P., (2009): Emergency endoscopy for upper gastrointestinal bleeding in patients with coronary artery disease. Am J Emerg Med. 27(7):802-9

Türkay C., Erbayrak M., Bavbek N., Yenıdünya S., Eraslan E., Kasapoğlu B., (2011): *Helicobacter pylori* and histopathological findings in patients with dyspepsia. Turk J Gastroenterol 22(2):122-7.

Vafaeimanesh J., Hejazi F., Damanpak V., Vahedian M., Sattari M., Seyyedmajidi M. (2013): Association of *Helicobacter pylori* infection with coronary artery disease: Is *Helicobacter pylori* a risk factor? The Scientific World JournalVolume 2014, Article ID 516354, 6 pages

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

Van den H. P.C., Van people N.M., Feskens E.J., Van der Kuip D.A. and Grobbee D.E. (1996): Blood pressure and risk of myocardial infraction in elderly men and women, the Rotterdam study, Journal of Hypertension. 17: 1373-1378.

Van Zwet, A.A., Thijs J.C. and Kooistra-Smid A.M. (1994): Use of PCR with feces for detection of Helicobacter pylori infections in patients. J Clin Microbiol, 32: 1346–1348.

Vareki S. M., Zarkesh-Esfahani H., Behjati M. (2013): *Helicobacter pylori* Evasion of the Immune System Could Establish an Inflammatory Environment That Potentially Induces the Development of Coronary Artery Disease. Jundishapur J Microbiol. 6(3) 243-247

Vats S., Sambyal Va. and Bhanwer A. J. S. (2013): Genetic Links between Coronary Artery Disease and Type 2 Diabetes. Human Biology Review (ISSN 2277 4424) 2 (2).

Veev A., Naki D., Mr|en A., Mirat., Balen S., Rui A., Per V., Soldo I., Matijevi M., Barbi J., Matijevi V. and Radanovi B. (2007): *Helicobacter pylori* Infection and Coronary Artery Disease. Coll. Antropol. 3: 757–760

Vibergsson S., Siguardsson G., Sigvaldason H. and Sigfusson N. (1998): Coronary heart disease mortality amongst non-insulin-dependent subjects in Iceland, the independent effect of diabetes, The Reykjavik study 17-years follow-up, Journal of Internal Medicine. 244: 309-316.

Vivek P. Singh, V. Ramesh, Sonal S., Nakul S., Satyendra T., and Suraksha A. (2006): Cardiovascular Risk Factors in North Indians: A Case-Control Study. American Journal of Biochemistry and Biotechnology 2 (1): 19-24

Wannamethee S.G., Shaper A.G. And Alerti K.G. (1997): Physical activity, metabolic factors analysis among Egyptian patients who underwent coronary artery bypass surgery, Texas Heart Institute Journal. 24: 204-208.

Wannamethee SG, Shaper G. (2003): Physical activity and cardiovascular disease, Semin Vasc Med. 2: 257–265.

Washio M., Sasazuki S., Kodama H. and Yoshimasa K. (2001): Role of hypertension, dyslipidemia and diabetes mellitus in the development of coronary arthrosclerosis in Japan, Japanese Circulation Journal. 65: 731-737.

Weinstein R. Amy, Howard D. and Kathryn M. (2008): The joint effects of physical activity and body mass index on coronary heart disease risk in women, Archive of Internal Medicine. 168: 884-890.

William Ng, Wong ., Chen ., Tse ., Lee P., Lai K., Li S., Matthew Ng, Lam k., Cheng X., Lisa S., Helen P. and William B. (2006): Overweight and obesity as

determinants of cardiovascular risk, Journal of American Medical Association. 162: 1867-1872.

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

Woodward M., Brazi F., Feigin V. and Gue D. (2007): Association between high-density lipoprotein cholesterol and both stroke and coronary heart disease in the Asia pacific region. European Heart Journal, 28: 2653-2660.

World Health Organization (2007): World health report. http://www. Who.int/whr/en/index.html. (Accessed on 3/9/2013)

Wu T, Chien K., Lin H., Hsu H., Su T., Chen M. and Lee Y. (2013): Total white blood cell count or neutrophil count predict ischemic stroke events among adult Taiwanese: report from a community-based cohort study. BMC Neurology2013, 13:7

Yamaoka Y., Kita M., Kodama T., Sawai N., Kashima K., Imanishi J., (2002): Induction of various cytoquines and development of severe mucosal inflammation by cagA gene positive *Helicobacter pylori* 656 Rev Esp Cardiol;55(6):652-6 130

Yao H., Bin J., Jie W. and Kang F. (2007): BMI versus the metabolic syndrome in relation to cardiovascular risk in elderly Chinese individuals. Diabetes Care Journal, 30: 2128-2134.

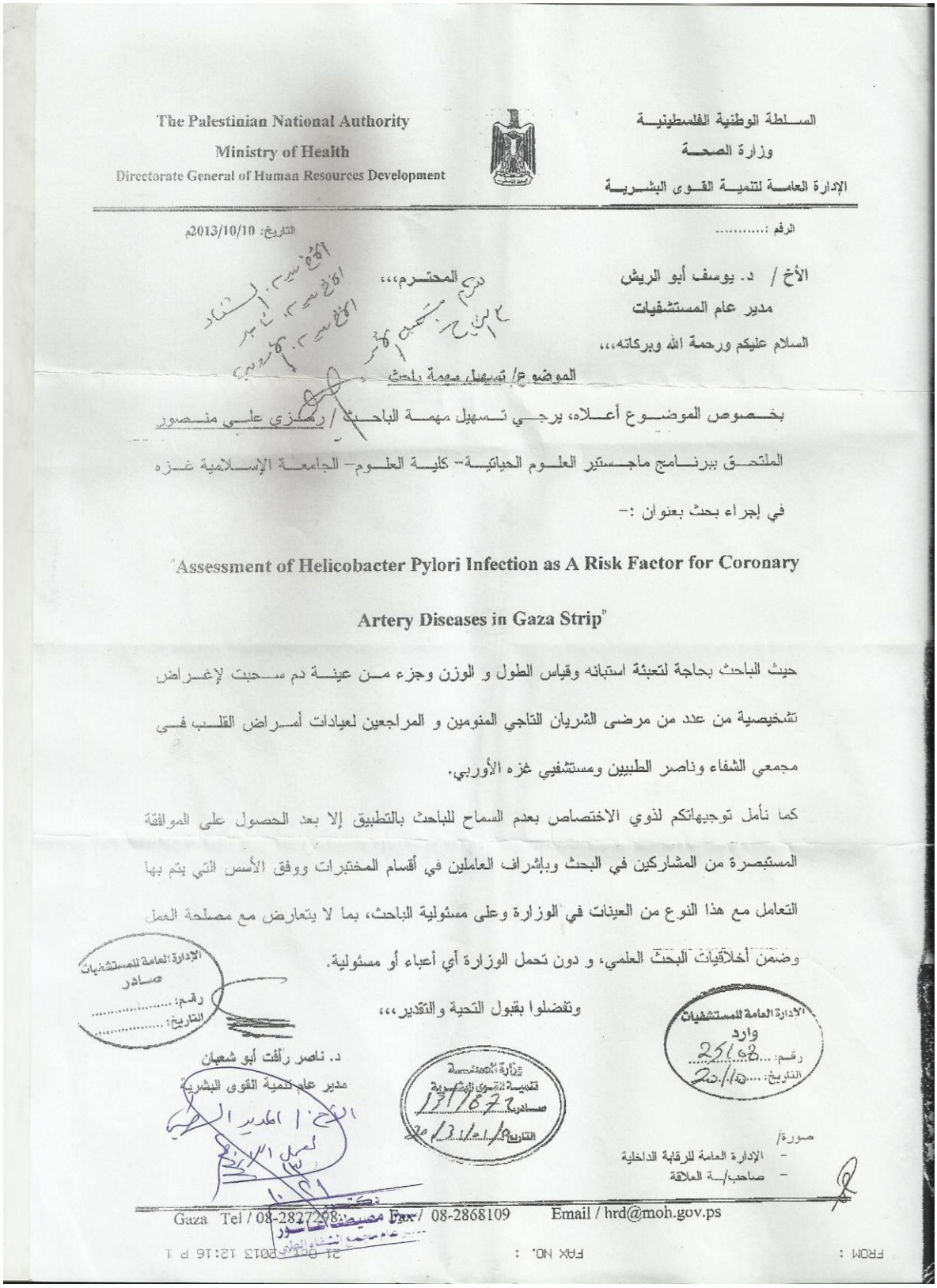
Zade S. M., Eishi A, Behrozian R, Rahimi E., (2009): Relationship between *Helicobacter pylori* infection and cardiac syndrome X. Shahrekourd Univ Med J; 11: 58-63.

Zahidullah M., Aasim M., Khan I., Muhammadzai H., Shah M. A., Ali N., Mohammad A., Muzahir A., Rehman M. (2012): Evaluation of patients with

Coronary Artery Disease for major modifiable risk factors for Ischemic Disease. J Ayub Med Coll Abbottabad; 24(2)

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, Gastroenterol Res Pract

(Annex 1)



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

السلطة الوطنية الفلسطينية
وزارة الصحة
الإدارة العامة لتنمية القوى البشرية

الرقم :.....

التاريخ: 2013/10/10م

الأخ / د. يوسف أبو الريش
مدير عام المستشفيات
السلام عليكم ورحمة الله وبركاته،،،

الموضوع/ تسهيل مهمة باحث

بخصوص الموضوع أعلاه، يرجى تسهيل مهمة الباحث / رمزي علي منصور الملتحق ببرنامج ماجستير العلوم الحياتية- كلية العلوم- الجامعة الإسلامية غزه في إجراء بحث بعنوان :-

"Assessment of Helicobacter Pylori Infection as A Risk Factor for Coronary Artery Diseases in Gaza Strip"

حيث الباحث بحاجة لتعبئة استبانه وقياس الطول و الوزن وجزء من عينة دم سحبت لإغراض تشخيصية من عدد من مرضى الشريان التاجي المنومين و المراجعين لعيادات أمراض القلب في مجمعي الشفاء وناصر الطبيين ومستشفيي غزه الأوربي.

كما نأمل توجيهاتكم لذوي الاختصاص بعدم السماح للباحث بالتطبيق إلا بعد الحصول على الموافقة المستبصرة من المشاركين في البحث وبإشراف العاملين في أقسام المختبرات ووفق الأسس التي يتم بها التعامل مع هذا النوع من العينات في الوزارة وعلى مسئولية الباحث، بما لا يتعارض مع مصلحة العمل وضمن أخلاقيات البحث العلمي، و دون تحمل الوزارة أي أعباء أو مسئولية.

وتفضلوا بقبول التحية والتقدير،،،

الإدارة العامة للمستشفيات
صادر
رقم: .....
التاريخ: .....

الإدارة العامة للمستشفيات
وارد
رقم: 2562
التاريخ: 20/10/13

وزارة الصحة
تنمية القوى البشرية
صادرة
رقم: 1371622
التاريخ: 30/10/2013

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

FROM : FAX NO. : 21 Oct 2013 12:16 P 1

(Annex 2)

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

كلية العلوم

التاريخ: 2013/10/8م

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

الموضوع / موافقة على بحث

نرجو من سيادتكم الموافقة على البحث المقدم من قبل الباحث / ***رمزي علي منصور***، وذلك ضمن برنامج ماجستير العلوم الحياتية التابع لكلية العلوم – الجامعة الإسلامية.

وهو بعنوان:

Assessment of ***Helicobacter pylori*** Infection as a risk factor for Coronary Artery Diseases in Gaza Strip.

سيجرى البحث في بداية أكتوبر 2013 .

لكم منا جزيل الشكر والامتنان،،،

- مرفق لكم نسخة من البحث.

منسق برنامج ماجستير العلوم الحياتية
د.محمد فؤاد أبوعودة

Islamic University P. Box 108 Rimal Gaza Palestine
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(Annex 3)

Questionnaire

Case control study Questionnaire for Assessment of *Helicobacter pylori* Infection as a risk factor for Coronary Artery Disease in Gaza strip

أخي المواطن الكريم /أرجو مساعدتنا في إتمام ههذه الدراسة (بحث ماجستير تحاليل طبية / الجامعة الإسلامية) و التي تختص بمرضى شريان القلب التاجي, حيث أن هدفنا الوقوف على مسبباته, و خاصة علاقته بالجرثومة الملوية البوابية (*H. pylori*). وذلك للحد من مضاعفاته.

Patients and controls Questionnaire

I. Sociodemographic data:

- Name: ..... Serial No.:.....
- Age:.....
- Address:.....Tel. No.: .....
- **Education**

  ☐ University ☐ Secondary school ☐ Preparatory school

  ☐ Primary school ☐ Illiterate

- **Employee** ☐Yes ☐No
- **Average family income per month (shekel)**

☐Less than 1000 shekel ☐ 1000-2000 ☐ More than 2000 shekel

- **Smoke cigarettes** ☐Yes ☐No
- **Physical activity** ☐Yes ☐No
- **Diet** ☐Yes ☐No
- **Family history of CAD** ☐Yes ☐No

II. Clinical data

- Duration of CAD (Years) for patients: .....
- Compliance of medication or treatments: ☐ Yes ☐No
- Has diabetes: ☐Yes ☐No
- Has hypertension: ☐Yes ☐No

III. History of *H. pylori* infection

- Gastritis ☐ Yes ☐No
- Peptic ulcers ☐ Yes ☐No
- Cancers of the esophagus and stomach ☐ Yes ☐No

IV. Anthropometric measurements

- Height (cm): ……..Weight (kg):…….
- Body Mass Index:
  …….

Agreement: I agree to complete this questionnaire concerning my health statement and for blood collection for laboratory analysis.

أنا موافق على تعبئة هذا الاستبيان الذي يتعلق بصحتي و اخذ عينة من الدم اللازمة لعمل الفحوصات المخبرية.

التوقيع: .....

التاريخ: .....

شكرا لكم على حسن تعاونكم

الباحث/ رمزي علي منصور