Risk Factors Associated with Coronary Artery Disease in Gaza

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هذا خلق الله فآروني لما خلق الذين ظلوا دونه بل الظلام فضلًا مبينًا

(لسان: 11)

كما قال رسول الله (سلام الله عليه وسلم):

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Khwiter S.                                Samy Hassan Khwiter                                 July. 2009

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ABSTRACT:

Coronary Artery Disease (CAD) remains the first killer and common silent disease in the world. The lipid profile plays the essential role in CAD development via atherogenesis process by depositing inside coronary arteries wall with lipid oxidation, which leads to artery narrowing then blockage. Recent studies showed inverse association between serum bilirubin and CAD development, although it involves endogenous anti-oxidant byproduct as HDL role. Our study aims to estimate the association of lipid profile, other risk factors and serum bilirubin with CAD development. Blood samples were taken from cross-sectional sample (n=94) of CAD inpatients (68 males and 26 females) recorded at the period of 1/6/2008 to 16/8/2008 at El-shefaa hospital of Gaza. The patient history of age, sex, BMI, diabetic, hypertension, smoking, physical activity, stress, working and family history were collected by questionnaire, hospital administration and nursing data in coordination with the Department Physicians. The lipid profile and serum Bilirubin were analyzed by spectrophotometer in the same hospital and private laboratory. SPSS version 15 was used as the tool for statistical analysis. Distribution of risk factor value of mean age was 57.3 (56 in males and 60.5 year in females). The middle age group (46-65 year) was higher than other groups (P<0.001). The mean BMI of total CAD patients was 28.7 (27.4 in males and 31.9 kg/m² in females) and females of normal weight group were higher than males (P=0.003). The mean cholesterol level >200mg/dl was 167 (166 in males and 177 mg/dl in females), and the distribution of high risk group was 24.5% (26.5% males and 29% female). The mean of triglyceride level >150mg/dl was 163 (170 in males and 164 mg/dl in females), and the distribution of high risk group was 41.5% (47.1% males and 73.7% females), the males were higher than females (P<0.05). The mean of HDL level >40mg/dl was 35 (35 in males and 33 mg/dl in females) and the distribution of lowered group was 72.3% (70.6% males and 76.9% females). The calculated LDL level >160mg/dl was 100 (97 males and 108 mg/dl females) and the distribution of high risk group was 19.1% (20.6% males and 15.4% females), the high total and direct bilirubin concentration group was (91.5% and 84%). The CAD under risk total cholesterol to HDL ratio (>4:1) was 62.8% (61.8% males and 65.4% females), CAD under risk LDL to HDL ratio (>3:2) was 39.3% (53.1% males and 20.4% females) and CAD under risk HDL to LDL ratio (<0.3) was 40.3% (61.4% males and 46% females). Distribution of risk factor value of hypertensive patients were 36.3% (35.3% males and 38.5% females), the diabetic was 38.3% (33.8% males and 50% females), the diabetic female
was higher than male. The nonphysical activity was 54 % (47.1% of male and 73% of female), sedentary or inactive was higher than weak and heavy activity and sedentary females were higher than males \( (P=0.001) \). The smoking patients were 44.7% (60.3% males and 3.8% females). The life stress patients were 30.9 % (26.5% males and 42.3% females), stress of female was higher than male \( (P=0.01) \). The workers were 60% (80.9% of male and 3.8% of female), distribution of workers were higher than non workers \( (P=0.03) \) and male workers were higher than female workers \( (P=0.001) \). The family history was 29 % (32.4% males and 19% females).

**Key words:** Atherogenesis, Coronary Artery Disease, Gaza strip and Lipid profile.
عوامل الخطورة المرتبطة بمرض الالتهاب التاجي بقطاع غزة

ملخص الدراسة:

مرض الالتهاب التاجي يعتبر هذا المرض هو المرض الصامت والقاتل الأول في العالم، حيث أن أشكال الدهون تلعب دوراً أساسياً في تطور المرض عن طريق عملية ترسب الدهون داخل جدار الشريان التاجي (atherogenesis) ثم أكتسبتها مما يؤدي إلى ضيق في الشريان ثم انسداد. أيضاً كثيراً من الدراسات الحديثة وجدت ارتباط عكسي بين تركيز bilirubin ونمو المرض، حيث أنه يشارك بعمل مضادات الأكسدة مثل الكولسترول الجيد مما يقلل من نمو المرض.

إن هذه الدراسة تهدف إلى تقديم الارتباط بين أشكال الدهون وتركيز bilirubin وغيرها من عوامل الخطورة مع نمو المرض. حيث تم الحصول على عينات الدم من عدة قطاعات عشوائية وكان حجمها 94 مريضاً يخضعون من انسداد الشريان التاجي وتوزعت العينة إلى (68 من الذكور و 26 من الإناث) والتي سجلت من فترة 1/6/2008 إلى 16/2008 من مستشفى الشفاء في قطاع غزة. كما تم الحصول على بيانات المرضى من العمر والجنس ومؤشر كتلة الجسم والسكري وارتفاع ضغط الدم والتدخين والنشاط البدني والإجهاد والعمل وتاريخ الأسرة المرضي من خلال الاستبيان، وسجلات قسم التمريض، وبيانات إدارة المستشفى بالتنسيق مع الأطباء المختصين. حيث تم الفحص بواسطة جهاز التحليل الطيفي في مختبر المستشفى ومختبر خاص لأشكال الدهون وتركيز bilirubin.

استخدمت أداة التحليل الإحصائي SPSS 15. حيث كان توزيع عوامل الخطورة لأعمار المرضى بقمة 57.3 (أعمار الفئات (65-60 سنة للإناث)، وكانت التوزيع الفردي المتنوعة (65-60 سن) هي الأعلى من غيرها من الفئات (P<0.001)، ومؤشر كتلة الجسم المترغبة كان 28.7 (P=0.003). ومستوى الكولسترول <200 ملغم كان 167 (166 للذكور و 177 للإناث) أما توزيعه في عينة مرضي المجموعة عامة الخطورة كان 24.5٪ (26.5٪ بالذكور و 29.7٪ بالإناث)، ومستوى الدهون الثلاثية <150 ملم كان 163 (170 للذكور و 164 للإناث) أما توزيعه في عينة المجموعة عامة الخطورة كان 47.1٪ (47.1٪ بالذكور و 73.7٪ بالإناث) في حين أن نسبة توزيع الذكور كانت أعلى من الإناث (P<0.05). أما الكولسترول الجيد بمستويو 40ملغم كان 35 (35 للذكور و 33ملغ للإناث) و توزيعه لمرضي عينة المجموعة المنخفضة المستوى كان 72.3٪ (70.6٪ بالذكور و 76.9٪ بالإناث)، ومستوى الكولسترول السبي المقصوب بمستوي 160ملغم كان 99.7٪ (97 للذكور و 108ملغ للإناث) أما توزيعه لعينة المجموعة عالية الخطورة كان 19.1٪ (20.6٪ بالذكور و 15.4٪ بالإناث). التوزيع الإجمالي للمباشر لمجموعة تركيز bilirubin المترغب كان (91.5٪ و 84٪).
و تتوزع عينة المجموعة علية الخطورة لنسبة الكولسترول على الكولسترول الجيد (CHL) كانت 62.8% (61.8% بالذكور و 65.4% بالإناث)، وتوزيع المجموعة علية الخطورة لنسبة الكولسترول السبيسي على الكولسترول الجيد (HDL) كانت 39.3% (1.53% بالذكور و 20.4% بالإناث)، وتوزيع المجموعة علية الخطورة لنسبة الكولسترول الجيد على الكولسترول السبيسي كانت 40.3% (61.4% بالذكور و 46.8% بالإناث). كما كانت قيمة التوزيع لمرضى ارتفاع ضغط الدم 36.3% (35.3% بالذكور و 38.5% بالإناث)، وقيمة التوزيع لمرضى السكري كانت 38.3% (33.8% بالذكور و 50% بالإناث) لكن نسبة الإناث كانت أعلى من نسبة الذكور، وتوزيع ذو النشاط البدني القليل كانت 54% (47.1% بالذكور و 73% بالإناث) كما أنه لوحظ أن متوسط النشاط البدني القليل 70% أعلى من متوسطي وعالي النشاط، كما تبين أن نسبة المجموعات النشاط البدني القليل للإناث أعلى من نسبة الذكور 44.7% (P=0.001) (60.3% بالذكور و 3.8% الإناث)، أما توزيع المرضى ذو الإجهاد النفسي كانت 30.9% (26.5% بالذكور و 42.3% بالإناث) بينما لوحظ أن توزيع نسبة الإناث كانت أعلى من نسبة الذكور (P=0.01)، وتوزيع المرضى العاملين 60% (80% بالذكور و 3.8% بالإناث) و توزيع نسبة العاملين أعلى من نسبة غير العاملين (P=0.03) كما كان معدل توزيع نسبة الذكور العاملين أعلى من نسبة الإناث العاملين (P=0.001).

أما توزيع المرضى ذوي تاريخ مرضى بالعائلة كانوا 29% (4.4% بالذكور و 19% بالإناث).

الكلمات المفتاحية: تصلب الشرايين، مرض شرايين القلب، قطاع غزّة، صور الدهون.
Dedication

To my beloved parents

To my wife Hanan

To my sons
Majd & Raghad

To my Brothers
Dr. Sameir, Moneer, Anwar, Mohammed-Ali and Maisraa

To all my Sisters.
Acknowledgment

I would like express my thanks to Dr. Abdalla Asaf Abed, my supervisor, who did not spare any efforts to overcome all the difficulties aroused during the theoretical and practical parts and for his constructive scientific advice. In the same time my deep appreciation to Dr. Mahmuod Sirdah, my co-supervisor, who did not spare any efforts to overcome all difficulties during the practical parts and for his helpful scientific suggestions and fruitful assistant.

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<tr>
<td>ABC1</td>
<td>Adenosine triphosphate -binding cassette transporter A1</td>
</tr>
<tr>
<td>ABCG1</td>
<td>Adenosine triphosphate--binding cassette transporter G1</td>
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<tr>
<td>apo A-I</td>
<td>Apolipoprotein A-1</td>
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<td>ATP</td>
<td>Adenosine triphosphate</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CAD</td>
<td>Coronary Artery Disease</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary Heart Disease</td>
</tr>
<tr>
<td>CETP</td>
<td>Cholesteryl ester transfer protein</td>
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<td>cGMP</td>
<td>Cyclic-guanosine monophosphate</td>
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<td>CHD</td>
<td>Coronary heart disease</td>
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<td>Co</td>
<td>Carbon Monoxide</td>
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<td>CVD</td>
<td>Coronary Vascular Disease</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene Diamine Tetra Acetic Acid</td>
</tr>
<tr>
<td>FH</td>
<td>Familial Hypercholesterolemia</td>
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<td>Heme oxygenase-1</td>
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<td>I/R</td>
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<td>(pre-I) HDL</td>
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<td>PAD</td>
<td>Peripheral Arterial Disease</td>
</tr>
<tr>
<td>ROS</td>
<td>Reduction/Oxidation</td>
</tr>
<tr>
<td>SMC</td>
<td>Smooth muscle cells</td>
</tr>
<tr>
<td>SO</td>
<td>Scavenger receptor</td>
</tr>
<tr>
<td>SPSS 15</td>
<td>Statistical Package for Social Science  Version 15</td>
</tr>
<tr>
<td>SR-B1</td>
<td>Scavenger receptor type B1</td>
</tr>
<tr>
<td>TC</td>
<td>Total Cholesterol</td>
</tr>
<tr>
<td>TG</td>
<td>Triglyceride</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very Low Density Lipoprotein</td>
</tr>
<tr>
<td>µl</td>
<td>Micro liter</td>
</tr>
<tr>
<td>O·</td>
<td>Single oxygen (free radical)</td>
</tr>
</tbody>
</table>
1.1 Overview

Coronary artery disease (CAD) has been remaining the first killer and the major cause of public health problems in the world, which is one of the most common causes of morbidity and mortality in different communities (1). Moreover, CAD is the main cause of death in the United States of America among human adults representing approximately one-third of all dead people, who are over the age of 35 years (1).

Coronary artery disease develops through narrowing of the coronary arteries which leads to death of portion of the heart muscle because of lacking of blood flow that supply oxygen and nutrition, and leads to heart attack. The coronary disease has two characteristics when compared with other organs disease. First, it is very commonly latent, which develops to an advanced stage before the patient notices any symptoms. Secondly, the number of symptoms attributable to heart disease is limited and it is similar to many different pathologies through a final common symptomatic pathway. The CAD mortality in North America and Western Europe in the recent decades has been successfully reduced by the treatment, while in contrast, it has increased in Asia and Eastern Europe (2).

Coronary artery disease development and progression is stimulated by environmental and/or genetic factors. The environmental factors include tobacco use, diabetes mellitus (D.M.), and hypertension (3). In most cases, CAD has a multifactorial genetic basis, involving a number of genes and environmental factors, which are interacting to determine whether or not the disease will develop as well as its severity (4).

Each of the biochemical processes has multiple constituents such as enzymes, receptors, and ligands, which are encoded by genes (5). Several biochemical processes participating in CAD development, include lipid and apolipoprotein metabolism, inflammatory response, endothelial function, platelets function, thrombosis, fibrinolysis, and blood pressure regulation (5).
It was shown in many previous studies that both control and patient groups differences in biochemical markers and other conventional risk factors analysed, such as systolic and diastolic blood pressures, dyslipidaemia, arterial hypertension, DM and a previous record of CAD in the family history are much higher in CAD patients than in controls. In addition, the average of high density lipoprotein (HDL) level lowered in the CAD group, and low density lipoprotein (LDL) level, total cholesterol and triglycerides were higher in the CAD patients (4).

Lipid profile plays the essential role of lipid deposition in artery wall and CAD development, by accumulating the LDL inside layers of artery wall, except HDL which has beneficial effects for a number of reasons by decreasing lipid oxidation after depositing in blood vessels, leading to retarding CAD development. Moreover, in other observational studies were shown that each 1-mg/dL decrease in plasma HDL concentration is associated with a 2% to 3% increased risk of CVD (6, 7). So, HDL is called "good cholesterol" according to its beneficial role in blood vessels by many mechanisms to prevent LDL from depositing on blood arteries, while LDL is called "bad cholesterol" due to its accumulation inside arteries.

Bilirubin exerts a strong antioxidant effects at physiological plasma concentrations against lipid oxidation in blood artery (3). While the heme oxygenase (HO-1) is a key enzyme of heme catabolism from red blood cells breakdown, which catalyzes the oxidative cleavage of heme which results in releasing CO, iron (Fe$^{2+}$), and bilirubin (8).

The lipid profile plays an important role in lipid deposition process in blood vessels but the bilirubin protects LDL from oxidative modification by potentiating the effect of exogenous antioxidants such as tocopherol or ascorbic acid (9). Therefore, the animal and human studies have substantiated the suggestion that bilirubin is a physiological antioxidant (10). Moreover, several studies had noted an inverse relationship between the presence of CAD and circulatory total bilirubin concentration (11).
To the best of our knowledge, this is the first study in Gaza strip investigating the significance of the association of lipid profile, bilirubin concentration and other risk factors with CAD development in Gaza population.

1.2 OBJECTIVES

1.2.1 Overall Objectives

- Overall objective is to assess factors associated with coronary artery disease in Gaza.

1.2.2 Specific Objectives

- To determine Lipid profile including Cholesterol, Triglyceride, HDL and LDL levels in CAD patients in Gaza.
- To determine total and direct bilirubin concentrations in CAD patients in Gaza.
- To find out the distribution of CAD factory among CAD patients in Gaza.
- To test the relation of lipid profile with CAD.
- To examine the relation of bilirubin with CAD.

1.3 Significance of the study

- Up to now, there is no any study that indicates the association of CAD risks factors (lipid profile and serum bilirubin) with CAD progression in Gaza. Therefore, this justifies the necessity to conduct the present study on the Gaza CAD patients.
- The estimation of distribution of CAD risk factors and lipid profile level with finding out the average of lipid profile in CAD development may give early alarms and early prognosis of CAD occurrence, and lead us to proceed for early prevention and precision treatment.
2.1 The coronary artery disease

Coronary artery disease also called as coronary heart disease (CHD), coronary atherosclerosis and Ischemic heart disease (IHD), which is a branch of coronary vascular disease (CVD) and a common form of heart disease. And, it is considered insidious and dangerous disease in the world, and the major source of morbidity and mortality in developed world (12). However, during the past 40 years, there had been an increasing awareness to evaluate the CAD risk factor in asymptomatic individuals (13). Acute myocardial infarction (AMI) is a major public health problem (14). And according to the published article in Emedicine website in 2005, by Vibhuti N Singh, referred to United States of American, in 1997, the atherosclerotic CAD accounting for 20% of all deaths. However, from 1987-1997, the death rate from CAD declined 24.9%. France and Mediterranean regions appear to have a lower incidence of CAD. This phenomenon partly explained by greater use of alcohol, with its possible HDL-raising benefit, and the Mediterranean due to consumption Mediterranean diet, which is less atherogenic. Also, Eskimos have a lower prevalence of CAD, which includes predominant use of monounsaturated fatty acids (15).

Coronary artery disease development is characterized by hard LDL-cholesterol (plaques) which are adiposities in the arterial wall of coronary with developing the buildup of blockage, leading to heart attack, ischemic condition, complete artery block or suddenly death. Two coronaries arise from the aorta, which is adjacent to the heart. The plaques can cause a tiny initial clot to form, which can obstruct the flow of blood to the heart muscle (as shown in fig. 2.1).

![Fig. 2.1 Coronary arteries and veins (16)](image)
The symptoms of CAD include:
1) Chest pain (angina pectoris) due to inadequate blood flow to the heart.
2) Heart attack, acute myocardial infarction (AMI), leads to death (14) due to sudden total blockage of a coronary artery.
3) Sudden death, due to a fatal rhythm disturbance.

2.1.1 Physiological structure of coronary
Heart is a muscular (myocardium) organ in all vertebrates animal, and it is considered an aerobic metabolic organ. Heart organ is responsible for pumping the blood through the blood vessels into whole body by repeated rhythmic contractions, while the coronary blood flow is closely coupled to myocardial ventilation oxygen consumption in normal hearts (15).

Myocardium depends almost completely on an aerobic metabolism, while the heart is accompanied by only small increases in myocardial oxygen consumption (17). The heart muscle is involuntary and it is found only in heart and needs energy by nutrition and oxygen coronary artery supply, which is almost exclusively depending on oxidation of substrates for generation of energy. However, it can develop only a small oxygen debt and still have enough energy to function its muscles normally (17).

Coronary arteries arise from aorta arteries and both are adjacent to the heart. The arterial coronary wall normally consists of three well-defined concentric layers that surround the arterial, the artery composed of the layer immediately adjacent to lumen called the intima, the middle layer known as the media, and the outermost layer comprises the arterial adventitia (3). Each of layers has a distinctive composition of cells and extracellular matrix (as shown in fig. 2.2).
2.1.2 Coronary artery disease development

2.1.2.1 Atherosclerosis "Arteriosclerosis"

Atherosclerosis word is of Greek origin and literally means gradual focal buildup and accumulation of lipid and fibrous tissue in plaques in the wall of arteries. (i.e., ATHERE [gruel]) and thickening of arterial intima (i.e., sclerosis [hardening]) (17). Atherosclerosis is the CAD or coronary artery atherosclerosis refers to the presence of atherosclerotic changes within the walls of the coronary arteries, it causes impairment or obstruction of normal blood flow with produced myocardial ischemia (17).

Initiated mostly from mixture of cholesterol, lipids, calcium, fibrous tissue, such as collagen, and other waste products, are depositing in the layers of the arteries. Buildup of fatty plaque called atherosclerosis, the progressive narrowing of the arteries with nourishes muscle of heart and can lead to progress of chemical and structural injury to the blood vessels in critical organs as the heart, brain, and kidney (19) (Fig. 2.3).

The genetic atherosclerosis such as a familial hypercholesterolemia (FH) is associated with mutations in genes, as well as mutations in some genes that lower HDL levels, such as the adenosine triphosphate–binding cassette transporter A1 (ABC1) canal is known to cause accelerated atherogenesis. Atherosclerosis developed by a multifactorial from genetics and environmental factors that play a role in the pathophysiology of the disease. On the other side, the environmental factors such as cigarette smoking, arterial hypertension and dietary cholesterol consumption are associated with an increased risk of CAD. In addition, the atherosclerosis more related to lipid profile such as cholesterol, triglyceride, HDL and LDL depending on several epidemiological and genetic studies were confirmed the association between elevated HDL
level and protection against atherogenesis (20). Therefore, the process of lipid deposition in the wall of blood vessels called atherogenesis.

2.1.2.2 Atherogenesis

Atherogenesis; is a process of arterial narrowing with atherosclerotic plaque development, whereas the accumulation of plaque in an artery wall is a chronic disease that begins early in life (5), with appears to be initiated and/or facilitated by chronic injury to the endothelium (5). The accumulated lesions progression take the form of an a cellular core of cholesterol esters bounded by a fibrous cap (5).

Since 1856, Virchow proposed that the atherosclerosis process starts with lipid transudation into the arterial wall and its interactions with cellular and extracellular elements (21). Therefore, different concepts supported the development of atherosclerotic lesions theory, depending on other observations. For these purposes, we must refer to some of these as follows (19):

- The response-to-injury.
- The response-to-retention.
- And Oxidative modification.

Therefore, the process of atherosclerosis proceeds via oxidative modification and developing as follows:

A. The Lipid oxidation: is a very complex process of LDL oxidation (22), and the critical step of atherosclerosis development. Many accumulated evidences that suggested the Oxidized low density Lipoprotein (OxLDL) plays an important role in early phases of atherogenesis via its proinflammatory properties (23) as shown in Fig. 2.4).

Fig. 2.4 Oxidation of LDL in atherogenesis (24)
In addition, the oxidative modification hypothesis focuses on the concept that LDL in its native state is not atherogenic (3). In contrast, when plasma LDL level increases, it leads to an increase in the adherence of circulating monocytes inside arterial endothelial cells. At the same time, the rate of entry LDL into intima layer increases, leading to higher steady state concentration of LDL in the intima layer (3).

Moreover, the exposure of the accumulated LDL in vascular cells to that medium which contains the transition metals will lead to a new modification of LDL into OxLDL inside the intima layer, which serves as a ligand in "Scavenger receptor" SO pathway (25).

At the same time the oxidative modification of lipoproteins in the vascular wall is essential for initiation and development of atherosclerosis (23), the consequence of oxidation, apolipoprotein B-100 lysine groups are modified with increases the net negative charge of lipoprotein particle (26). This modification of apolipoprotein B-100 renders LDL susceptible to macrophage uptake via a number of so-pathways producing cholesterol ester-laden foam cells (26), from normal circulating monocytes to cholesterol filled foam cell inside the intima layer.

It was reported that the OxLDL contributes to atherogenesis by the following (3):-
- Aiding the recruitment of circulating monocytes into the intimal space
- Inhibiting the ability of resident macrophages to leave the intima
- Enhancing the rate of uptake of the lipoprotein with leads to foam cell formation
- 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 (27).
In addition, each treatment hypothesis's includes LDL as a central element, the treatment focuses on the reduction of LDL cholesterol which is among the most effective means of treating atherosclerosis (3).

B. Monocytes accumulation and atherosclerotic artery: OxLDL is immunogenic, and autoantibodies and are commonly found in patients (28), which leads to foam cells accumulating in the area. Therefore, OxLDL had been implicated to be involving in early atherogenesis (29).

Although, the macrophage attempts to remove injurious materials (e.g. oxLDL) via scavenger receptors by forming the foam cells (12). Nevertheless, the accumulation of foam Cells (lipid-rich macrophage) form as nodular areas of lipid deposition that are also known as, fatty streaks and these represent lipid-filled macrophages (Fig. 2.5).

Low density lipoprotein compound bounds to LDL receptor, internalized, and transported through the endothelium. Low density lipoprotein initially occur in the subendothelial space and stimulate vascular cells to produce cytokines for recruiting monocytes, and further LDL oxidation. Then, soon after a lesion initiated, there is fragmentation of the internal elastic membrane and migration up of smooth muscle cells from the media into the intima, to continue developing atherosclerotic lesion (3).

Fig. 2.5 atherosclerosis progressive (30)
C. Plaque Rupture (Fissure): after the accumulated plaques of core lipid, thick fibrous filled with inflammatory cells as macrophage, T cells, and other differences in the morphology, this suggesting the vulnerable plaques may be weaker structurally and more likely to rupture in response to the physical forces of blood flowing inside swelling of artery wall (3). This contention supported by experimental data linking an increased content of macrophages in lesions to structural weakness (3). In addition the light microscopic appearance of a prototypical atherosclerotic plaque is depicted in plaques contain a central lipid core that is most often hypocellular and may even include crystals of cholesterol that have formed in the aftermath of necrotic foam cells (3).

D. Occulassive thrombus: is by platelets begins to accumulating at the site of a vulnerable coronary plaque, which plays a major role in the pathophysiology of AMI (14). Platelets begin attach to the vessel wall and initiate thrombotic occlusion of the coronary vessel. Besides thrombus formation in the epicardial arteries, and platelet microembolization and accumulation within the microcirculation of the ischemic myocardium to play a major role in microcirculatory arrest thus promoting tissue damage (14) by:

- thrombotic occlusion of an epicardial coronary artery at a disrupted, eroded atherosclerotic plaque,
- microembolization of atherothrombotic platelet-rich aggregates,
- platelet-mediated vasoconstriction,
- enhanced intravasal thrombus formation in the microcirculation and
- Platelet-mediated inflammatory reactions in the ischemic myocardium

Combination of these events will determine the degree of disease severity (14).

Platelets play a major role in coagulation of the blood in vessels, mainly due to retardation of blood flow. Therefore, the critical role for platelets in the acute manifestations of atherosclerosis supported by data that platelet inhibition yields a reduction in myocardial infarction and stroke (3). Continual a plaque lesion expansion with progressively the arterial narrow lumen and eventually limit blood flow, thereby causing tissue ischemia (3). Inevitably, it was thought that arterial occlusion would result and necrosis of the target organ would ensue.
It was reported that the AMI and crescendo angina, two cardinal manifestations of atherosclerosis, were associated with atherosclerotic plaque rupture and fissuring in the artery with compromised blood flow (31). This chain of events frequently results in heart attack or sudden death without warning.

**E. Thrombosis Progression:** In the past, a numerous studies have shown that platelets can adhere to the intact endothelial monolayer and substantially modulate endothelial cell function (14). Thus, under certain pathophysiological circumstances, endothelial denudation and exposure of subendothelial matrix are not required for platelet adhesion to the vascular wall (14). However, platelet aggregation is associated with manifest of oxidative stress as a burst of oxygen consumption and also be exposed to Reduction/Oxidation (ROS) process that arise from the vascular wall (3). In addition, platelets aggregate in response to exposed vessel wall collagen or local aggregates (e.g., thromboxane, adenosine diphosphate) (12).

Whereas adherent the platelets releases a variety of pro-inflammatory mediators and growth hormones and have the potential to modify signaling cascades in vascular cells, inducing the expression of endothelial adhesion receptors and the release of endothelial chemoattractants (14). Moreover, the platelets release substances that promote vasoconstriction and production of thrombin. In a reciprocating fashion, thrombin is a potent agonist for further platelet activation, and it stabilizes thrombi by converting fibrinogen to fibrin (12). While the plaques develop may become symptomatic when they are large and enough to restrict blood flow leading to tissue ischemia (5). And the acute coronary syndromes such as unstable angina, MI, and/or death suddenly occurs when unstable plaques rupture or ulcerate lead to platelet accumulation and activation, fibrin deposition, thrombus formation (5).
2.2. Assessment of coronary artery disease risk factors

Over the past 150 years there have been numerous efforts to explain the complex events associated with the development of atherosclerosis. Many previous studies confirmed that the CAD development has a strong relationship with many risk factors, therefore the risk factors are important to prognosis and prediction of CAD development. In addition, a number of clinical and laboratory variables have proven predictive of the incidence of cardiovascular disease, moreover, the observational study was referred to a strong and consistent main risk factor associated with cardiovascular disease (3). The Framingham Risk Score is one of the most widely used risk assessment methods for prediction of CAD risk, considers the established risk factors of gender, age, smoking, total cholesterol, LDL cholesterol, HDL cholesterol, and DM(5). The Framingham Risk Score underestimate CAD risk for individuals with a family history of CAD at younger ages, moreover does not consider family history as a risk factor (5).

2.2.1. Coronary artery disease risk factors

2.2.1.1. Controllable CAD risk factors

A. Hypertension: is defined as a systolic blood pressure in excess of 140 mmHg or a diastolic blood pressure above 90 mmHg (32). Uncontrolled high blood pressure can result in hardening and thickening of the coronary arteries, narrowing the channel through which blood can flow.

In addition, it was indicated that the elderly are particularly predisposed to hypertension, with up to 75% of people over 75 years of age qualifying for CAD diagnosis. There appears to be an approximately linear relation between blood pressure elevation and increased the incidence of atherosclerotic vascular disease (32).

In observation study it was referred that the hypertension was considerably more frequent among cases than control subjects. Therefore, the hypertension considers the main risk factor of CAD development in many populations (33).

B. Serum Hypercholesterolemia: defined as an increased in serum cholesterol level due to environmental or genetic factor. Many previous studies indicated that the prime causative and actual factor of the heart disease is high
cholesterol level (34). The association between lipid profiles and CAD development is determining via cholesterol levels. The cholesterol is the essential player of lipid profiles in atherogenesis process of CAD progressive, which is the main CAD indicator and CAD monitor. However, the hypercholesterolemia reported to be strongly associated with enhancing oxidative stress, via increased lipid peroxidation and tends to increase the susceptibility of LDL to oxidation (35). According to previous studies hypercholesterolemia is considered from the main risk factor of whole lipid profile. However cholesterol is in part dependent on age and sex, the serum cholesterol level between male and female not equalized, and during age decades was observed many differences in cholesterol levels between sex groups, also females have higher cholesterol levels than males until about age 20 year, and no difference between sex groups after age of 40 years (34).

The genetic relation is considered as one of risk factors, however the presence of genetic factor such as familial hypercholesterolemia (FH) is the first entity directly associated with the development of premature atherosclerosis and CAD. Familial hypercholesterolemia a heterozygotes genetic disorder, which is manifest a 2-5 fold elevation in plasma LDL cholesterol that is due to a functional impairment of the LDL receptor (3). In FH heterozygotes, 85% of individuals have experienced a MI by the age of 60, and this age reduced to 15 yr in patients homozygous for the disease (36). While the FH homozygote's demonstrate a 5-6 fold elevation in plasma cholesterol that produces precocious atherosclerosis (3).

Tangier disease is an autosomal codominant condition, characterized by essential absence of HDL cholesterol levels due to a defect in the ATP binding cassette transporter-1(37), which is impairs cholesterol efflux from cells (38).

C. Cigarette smoking: Cigarette smoke contains over 4,000 known components, which is considering one from the main risk factors of CAD development in the most of population. Many studies observed the presence of a strong association with the cigarette smoking and many other diseases, the cigarette smoking more linked to heart diseases. The nicotine constricts blood vessels, and carbon monoxide (CO) can damage their inner lining, making them
more susceptible to atherosclerosis. However in both animal and human models, several studies have demonstrated that the cigarette smoking exposure were associated with a decrease in vasodilatory function (39). Cigarette smoking could promote atherosclerosis, in part, by its effects on lipid profile. Smokers have significantly higher serum cholesterol, triglyceride, and LDL levels, but HDL is lowering in smokers than in nonsmokers (39). It was estimated that the smoking increases atherosclerotic disease by 50% and doubles the incidence of CAD (42 and 43). The author reported that the cigarette smoking might increase the risk of CVD as a general by lowering antioxidant concentrations and raising oxidized lipid and lipoprotein concentrations (42).

D. Physical activity: The regular exercise or physical activities consider the most important and the beneficial treatment methods for people that under CAD risk (43). In the United States of America (USA) an epidemiological research on physical activity revealed that the physical inactivity appears to be a more important CAD risk factor. Therefore, the physical activity has been listed as the first priority area for the newly released "Healthy People 2010" objectives, of the USA Department of Health and Human Services (43).

The physical activity retarding CAD development via reduces VLDL levels, raises HDL levels, sometime decrease LDL level, and lower blood pressure, reduce insulin resistance, and favorably influence cardiovascular function with and coronary blood flow, in the same time effectively will be reducing all of other risk factors (12). However, the first-line therapies for all lipid and nonlipid risk factors are associated with weight reduction and increased physical activity (12).

E. Diabetes Mellitus (DM): is defined as an increased of blood glucose level (hyperglycemia). Recent and previous studies observed the presence of a strong association between CAD development and DM, therefore the DM is consider one of the main risk factor of CAD development (44). In DM patients the risk of coronary atherosclerosis are elevated three-to five fold greater than in non diabetics despite controlling for other risk factors (45).
F. BODY MASS INDEX (BMI):

- **The body mass index**: is a key index measurement for relating a person's body weight to their height. It had been used by the World Health Organization (WHO) as the standard for recording obesity statistics since the early 1980s. The BMI invented between 1830 and 1850 by the Belgian polymath Adolphe Quetelet during the course of developing "social physics" (46).

Body mass index is a statistical measurement that compares a person's weight and height, though it does not actually measure the percentage of body fat, but it is a useful tool to estimate a healthy body weight based on how tall a person is. Due to its ease of measurement and calculation, this is the most widely used diagnostic tool to identify weight problem. Body mass index was defined as the individual's body weight divided by the square of his height. While the calculation of BMI equation is by dividing the person's weight (in kilograms) at their height (in meters squared) (35), and the formulas universally used in medicine produce a unit of measure of kg/m$^2$.

BMI can also be determined by using a BMI chart (fig. 2.6).

\[
\text{BMI} = \frac{\text{weight (kg)}}{\text{height}^2 (\text{m}^2)}
\]

Fig. 2.6 BMI chart (47)

According to many previous studies, it was adopted the cut point of BMI $\geq$30 kg/m$^2$ as a surrogate marker for central obesity, due to the waist circumference was not available for the premature familial CAD cases (33). The overweight was defined as BMI 25–30 kg/m$^2$ for males and females but the obesity was defined as BMI $\geq$ 30 kg/m$^2$ (2) (Table 2. 1).
The elevated body-mass index associated with several risk factors for CAD, including hypertension, dyslipidaemia, and D.M. and other factors (48).

The prevalence of all features of the metabolic syndrome increased with increasing BMI, while the BMI not independently of associated with CAD risk (33).

- **Obesity or overweight**: is defined as an excess in body weight with an abnormal high preponderance of body fat. The obesity has become a major public health issue in the United States (48) and the prevalence of obesity in the developed world is increasing at an alarming rate (3). Since 1970, the prevalence of overweight among children between ages 2 to 5 years has been doubled, while among children and adolescents between ages 6 to 19 years it has been tripled. Recently studies considered more than 9 million children and adolescents (17%) are being overweight (48).

<table>
<thead>
<tr>
<th>BMI range - kg/m^2</th>
<th>Category</th>
<th>Waist less than or equal to 40 in men) or 35 in women</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.5 or less</td>
<td>Underweight</td>
<td>N/A</td>
</tr>
<tr>
<td>18.5 - 24.9</td>
<td>Normal</td>
<td>N/A</td>
</tr>
<tr>
<td>25.0 - 29.9</td>
<td>Overweight</td>
<td>Increased Risk</td>
</tr>
<tr>
<td>30.0 - 34.9</td>
<td>Obese</td>
<td>High Risk</td>
</tr>
<tr>
<td>35.0 - 39.9</td>
<td>Obese</td>
<td>Very High Risk</td>
</tr>
<tr>
<td>40 or greater</td>
<td>Extremely Obese</td>
<td>Extremely High Risk</td>
</tr>
</tbody>
</table>

Table 2.1 BMI ranges (50)

The relationship of obesity with CAD is running in the same line with some other risk factors, as a number of other risk factors for cardiovascular disease, such as hypertension, low HDL cholesterol and DM, often coexist with obesity (50). However, the exact mechanisms explain this phenomenon is controversial, while today the relationship between obesity and cardiovascular disease has become of considerable concern (3).

**J. Stress**: is the way of metabolic body reacts to change, includes our mental, emotional, and physical responses to the pressures of everyday life, with change is a natural and normal part of life, therefore a moderate amount of stress is part of normal living (49). Stress may be good by acting body motivate and more productive, while the strong stress response is a harmful with set the
body for general poor health as well as specific psychological or physical illnesses like heart disease or infection.

The stress can be acute or long-term chronic, the acute stress is a "fight-or-flight" reaction to an immediate threat. Triggers of acute stress include crowds, noise, and dangerous situations. The chronic stress requires suppressing natural "fight-or-flight" reaction over hours, days, or even years. The chronic stress triggers include demanding jobs, family problems, marital problems, money worries, or feelings of inadequacy or loneliness (49).

In body system the acute stress mechanism will react with stimulators by inducing major body organs such as the brain organ to send out a various of signal hormones includes cortisol and adrenaline hormone, the immune system to prepare its system for the attack, the heart organ to increase blood pressure, the lungs to increase the breathing and become more rapid to take-in more oxygen in lungs, the circulatory system to increase blood flow 300% to 400% to get the muscles ready for any added demands and the spleen to release more red blood cells. All these organs will be prepared and ready for kick into high gear (49). Therefore, unrelieved that the stress mechanism in body system may damage the arteries as well as worsen other risk factors for CAD progression (Table 2.2).

<table>
<thead>
<tr>
<th>Table 2.2 Stress and the heart (51)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>What stress does</strong></td>
</tr>
<tr>
<td>narrow the arteries</td>
</tr>
<tr>
<td>Increases the blood pressure</td>
</tr>
<tr>
<td>Increases the heart rate</td>
</tr>
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2.2.1.2. Uncontrollable CAD risk Factors

A. **Age:** is among the most important risk factors for predicting incident cardiovascular disease. Based on previous experience studies in the United States the average risk of developing cardiovascular disease for a 30-34 year old male is 3%, this number raises some sevenfold to 21% for a comparable individual aged 60–64 years (52). The exact importance of age-related risk compared with other cardiovascular disease risk factors illustrated by the **Framingham Heart Study** that has resulted in a 14-point scoring system to predict incident 10-year cardiovascular disease. In this system, the increasing risk characterized by a higher score, up to 7 points can be attributed to age alone (3).

In addition, the cumulative risk for CAD in males by age 70 is 35% and by age 90 is 49% (5). While the women typically develop CAD about 10 years later then men with a cumulative risk of 24% and 32% by ages 70 and 90, respectively (5). Therefore they considered a disease of advancing age is approximately 15% of cases are diagnosed before age 65 (5). While at young ages the disability and mortality from CAD is particularly devastating to families and has a substantial impact on our economy (5). In addition, the individuals with genetic predisposition to atherosclerosis are at the greatest risk for developing CAD, especially at early ages, and they have the most to gain from preventive interventions (5).

B. **Family history of early heart disease:** The premature CAD is known to have a particularly strong genetic component. Previous data suggested that the genetic factors are more likely to affect young rather than old people (4). They conducted a population subdivision, analyzing separately individuals who developed CAD before the age of 45 (4).

In statistical studies done in 2003 in USA, an estimated 12,600,000 Americans have CAD, estimated 650,000 Americans will have their first heart attack and another 450,000 will have a recurrent event (5).

C. **Gender or sex:** is important risk factor in incidence of CAD and many studies noted difference between male and female in CAD distribution among population. Also the distribution differs from population to other. However,
numerous observational studies have indicated that males exhibit excess risk for cardiovascular disease compared with age-matched women (53). A woman’s risk rises once she enters menopause, this speculation depends on estrogens hormone, and will offer a protective effecting role to women body system, and cardiovascular disease accelerates in women after menopause. However, this speculation has been difficult to substantiate, as the treatment with estrogen has not reduced the incidence of cardiovascular disease of postmenopausal women (54). The estrogens have biphasic effects on hepatic cholesterol metabolism (3). The strength of the relationship between low HDL levels and increased CVD risk also is significant in elderly individuals and may be greater in women than in men (3). Alternatively, some of this apparent protection could be due to the fact, that women exhibit relatively higher concentrations of HDL cholesterol than do age-matched men (3).
2.3. The association of Lipid profile with coronary artery disease development

The elevated levels of serum total cholesterol, triglyceride and LDL and low levels of HDL is called dyslipidaemia, where is a major risk factor for CVD. However all the components are associated with increased incidence of CAD (56).

Lipid profile (Cholesterol, Triglyceride, and LDL) are the essential players compounds in CAD developments steps, except HDL which retards CAD development. According to other studies of lipid profile with CAD relationship, multiple epidemiologic studies have established a low level of HDL as an independent risk factor for CVD (55). Moreover, Framingham Heart Study reported 43% to 44% of coronary events occurred in persons with HDL levels less than 40 mg/dL (56).

The study observation was done by the author referred that the CAD risk increased the substantially with TG elevations begin at 200 mg/dl which is a clinical significance, because this is a common and relatively mild lipid abnormality and the risk was independent of other features of the metabolic syndrome (56). In addition the risk of CAD was approximately doubled with either TGs >200 mg/dl or HDL-cholesterol <40 mg/dl (33). Moreover, the presence of both was associated with a four-fold increase in risk (33). The high prevalence of familial TG elevations among premature CAD patients further illustrates the importance of hypertriglyceridemia as a CAD risk factor (33).

Another much recently observational study from Jordan kingdom referred that the chronic CAD patients had higher TG and lower HDL levels compared with those without CAD, also it was founded those CAD patients had significantly higher TG and TC, and lower HDL levels than individuals with no CAD (56). In addition it was reported that the high levels of serum cholesterol and TG were present in at least half the participants, however only one fifth of CAD patients had hypercholesterolemia, and about half of them had elevated TG (56). The prevalence of low HDL-C was 45% in men and 23% in women, similar to that in Lebanon (47% in men and 15% in women) but lower than that in the Islamic Republic of Iran (57% in men and 47% in women) (56), higher than that in
Saudi Arabia (28%) (56). The United States of America is (18% in men and 6% in women) (56).

2.3.1. Lipid profile and atherogenesis

The lipids and other fats circulate in blood via forming lipoproteins particles found in plasma transport lipids including cholesterol, spherical particles with a hydrophobic core contains TG and esterified cholesterol, and apolipoproteins on the surface which consist of:

- **Large**: apoB (B-48 and B-100) atherogenic
- **Smaller**: apoA, apoC-II, and apoE classified based on density and electrophoretic mobility (very-low-density lipoprotein (VLDL); LDL; Intermediate Density Lipoprotein; HDL; Lipoprotein-a) (57).

Lipid profile consists of cholesterol, LDL, IDL and TG which are the essential players compounds in CAD development forward steps, except HDL which is retarding and inhibit CAD development, by many mechanisms of action (Fig. 2.7).

The lipid transporting by the bloodstream in particles made of fats and proteins to travel throughout the blood stream, therefore via packets intermediate compound via forming chylomicron formed through extrusion of resynthesized TG from the mucosal cells into the intestinal lacteals. These particles called lipoproteins and were classified by their density into many type of lipoproteins (57), HDL, LDL, IDL, and VLDL, while the TG are another type of fat molecule in the blood (57). Moreover, The Helsinki Heart Study considered the chylomicronemia might confer low CAD risk despite very high TGs (33).
In addition, LDL does not aid in the transportation of cholesterol out of the body, instead it deposits cholesterol onto the vessel wall, due to LDL molecules contains much more cholesterol than HDL molecules (Fig: 2.7).

2.3.1.1. Total cholesterol (TC)

The cholesterol compound provided by external source via food intake or internal source via synthesized it in hepatocytes. The cholesterol compound is water-insoluble. Cholesterol is found in every cell wall in the body and plays a critical role in maintaining cell integrity, without it the cells would not be able to maintain their spherical shape (57). In addition, many hormones inside the body made from cholesterol (57). Moreover, it is considered the main source to synthesize HDL, LDL, IDL and VLDL.

2.3.1.2. High density lipoprotein, low density lipoprotein and very low density lipoprotein

Low density lipoprotein and HDL play the major role in atherogenesis process development or inhibition. It was found that the oxLDL have various biological effects on vessel walls, including stimulation of cytokine production, inhibition of endothelial cell vasodilator function, and stimulation of growth factor production, as well as providing mechanistic links between lipoproteins and the cell biology of atherosclerosis. These observations raise the more general possibility that abnormalities of the oxidation-reduction state in the vessel wall may be an important pathogenic mechanism in atherosclerosis (35), which lead to start the atherogenesis process in layers of vessel wall. Therefore, LDL is called the bad cholesterol.

On the other hand, the HO-1 gene induced by mildly oxLDL (59). Moreover, the LDL is increasing the response of HO-1 to oxLDL. This adaptive response contributes to the maintenance of vascular tone and potency in atherosclerotic vessels (60).

In contrast, HDL has a reverse relation with CAD development, which is acting as a mop to extract excess cholesterol deposited in blood vessel walls and delivering it back to liver for elimination through gastrointestinal tract, via many of metabolic pathways. The decrease of CAD severity was seen even
with HDL above 40 mg/dl (33). Angiographic and ultrasonographic data indicated that the lowering of levels of HDL were associated with risk and severity of CAD (7). On the other hand, epidemiological studies had associated the elevation of LDL levels with an increased risk for CAD. The Framingham Heart Study observed that the individuals with HDL concentrations of ≥ 60 mg/dL are protected against the development of CAD even in the presence of elevated serum LDL levels (20). In another study, HDL > 45% decreased the frequency of atherosclerosis. In addition low HDL and high TG were recognized as independent coronary risk factors, and these may potentially play a more important role in the pathogenesis of atherosclerosis in this region of the world than hypercholesterolemia (56). Therefore, HDL is called the good cholesterol.

Physiological protection mechanism of HDL

The beneficial effects of HDL are via protection through multiple pathways, which is including both reverse cholesterol transport and non–cholesterol-dependent mechanisms (61) (Fig. 2.8).

Reverse cholesterol transport: is involved in transfer of excess cholesterol from lipid-laden macrophages present in peripheral tissues to the liver via HDL, with subsequent catabolism of cholesterol or excretion into bile (62).

In the vessel wall the cholesterol ester stored in macrophages, then in macrophage converts cholesterol-ester to free cholesterol by cholesterol ester hydrolase (CEH), whereas the acyl-cholesterol acyltransferase esterifying the cholesterol within macrophages to form atherogenic foam cells (3). To decreases the accumulation of foam cells inside artery wells and transport it into outside wall, the liver and intestine synthesize lipid-poor apo A-I protein, which interacts with the transporter ABC1 cell canal located on the arterial macrophages surface, to transporting free cholesterol to extracellular lipid-poor HDL (3), Apo A-1 interacts with free cholesterol to form HDL. Thin interacts with other lipids via lipidation process of HDL particles, and will be generates nascent (pre-I) HDL (63).
Subsequently, lecithin-cholesterol acyltransferase esterifies free cholesterol within nascent HDL to produce mature \( \alpha \)-HDL particles (i.e., HDL\(_3 \) [smaller, more dense particles] and HDL\(_2 \) [larger, less dense particles]) (3). The mature HDL particles can further take up free cholesterol via the *macrophage adenosine triphosphate–binding cassette transporter G1*, the mature \( \alpha \)-HDL has at least two metabolic fates (3):

- **Direct pathway:** Cholesterol esters contained within HDL can undergo selective uptake by hepatocytes and steroid hormone–producing cells via the scavenger receptor type B1 and subsequent excretion into the bile (62).

- **Indirect pathway:** Cholesterol esters within HDL can be exchanged for TG in apolipoprotein B–rich particles (LDL and VLDL) through the action of *cholesterol ester transfer protein* (CETP) (3). Subsequently uptaking of apolipoprotein B rich in cholesterol esters by hepatic LDL receptors may be responsible for up to 50% of reverse cholesterol transport (62). Triglyceride-rich HDL can then undergo hydrolysis by hepatic lipase and endothelial lipase to form small HDL for further participation in transport (62).

**Non–cholesterol-dependent mechanisms:** On the other hand, HDL has other beneficial biological activities that may contribute to protective effects against atherosclerosis development (61) such as:

- antioxidant effects via counteracting LDL oxidation,
- anti-inflammatory effects,
- antithrombotic/profibrinolytic via reducing the platelet aggregation and coagulation effects and vasoprotective effects via facilitating vascular relaxation
- and inhibiting leukocyte chemotaxis and adhesion (61).

In general, the HDL and its components associated (including apo A-I, paraoxonase, platelet activating factor acetylhydrolase, and other antioxidant enzymes) exert an array of effects that may help prevent atherosclerosis and acute coronary syndromes (65).
Excess cholesterol stored in macrophages in arterial walls contributes to atherosclerosis.

In reverse cholesterol transport, cholesterol ester hydrolase (CEH) releases free cholesterol from cholesterol ester (CE) stores.

The ABCA1 transporter facilitate the efflux of cellular cholesterol to lipid-poor apo A-I to form nascent pre-β-HDL. Apo A-I is produced in the liver and intestine, and is also generated upon catabolism of mature HDL.

Lecithin-cholesterol acyltransferase (LCAT) esterifies free cholesterol in nascent pre-β-HDL to cholesterol ester, converting nascent pre-β-HDL to mature α-HDL (HDL2 and HDL3).

Interconversion of mature α-HDL subspecies (HDL2 and HDL3) can occur in the arterial wall and in plasma. These interconversions are mediated by hepatic lipase (HL), endothelial lipase (EL), and LCAT.

Indirect Pathway of Hepatic Cholesterol Uptake
Cholesteryl ester transfer protein (CETP) facilitates the exchange of CE in HDL for triglycerides (TG) in TG-rich apo B particles (LDL, VLDL).

Direct Pathway of Hepatic Cholesterol Uptake
CE is taken up via SR-B1 receptors on hepatocytes that recognize apo A-I as a ligand.

Cholesterol is catabolized and subsequently excreted into bile.

Fig. 2.8 Overview of physiological cholesterol HDL transport and HDL metabolism (66)
2.3.1.3. Triglyceride (TG)

TG is a member of chylomicron compounds that sharing in buildup LDL and HDL. Several lines of evidence suggest that the association of plasma TGs with CAD is complex, however despite this consensus, uncertainty persists regarding the strength and independence of plasma TGs as a CAD risk factor (33). In European studies elevation of plasma TG concentration become increasingly established as an independent risk factor for premature CAD (33).

The study of Prospective Cardiovascular Munster (PROCAM) reported that the CAD risk increased proportionately with TGs up to 800 mg/dl. The risk is associated with TGs >200 mg/dl and was dependent on concomitant low HDL or elevated LDL to HDL ratio (33).

In contrast, type III hyperlipidemia (also called familial dysbetalipoproteinemia) is defined by the accumulation in plasma of highly atherogenic, abnormal, cholesterol-enriched remnants of TG-rich lipoproteins that results from impaired removal of TG-rich lipoprotein remnants, and may associate with extreme CAD risk despite relatively modest TG elevations (33). Moreover, the high prevalence of premature atherosclerosis in coronary and other arteries is readily apparent from case series of patients with type III hyperlipidemia (33). The type III hyperlipidemia was found in 0.4% of men as in the general population (33). The moderate elevations in TGs seen with metabolic syndrome seem to be associated with moderate CAD risk (33).
2.4. Bilirubin

2.4.1. Bilirubin production and excretion

In adults, human, ~250 – 350 mg of bilirubin is produced daily, primarily through the breakdown of Hb in many organs but mainly in spleen (67). Nearly 80% of bilirubin is produced from Hb of red blood cells breakdown, by the HO-1 enzyme, which opens the heme (Fe-protoporphyrin IX) molecule ring, and liberating the product with forming the linear tetrapyrrole molecule biliverdin, and then it is reduced to bilirubin.

These processes all occur in the reticuloendothelial cells of the liver, spleen, and bone marrow. The bilirubin is transporting to the liver where it reacts with a solubilizing sugar called glucuronic acid, which is more soluble form of bilirubin (conjugated) is excreted into the bile. The bile goes through the gall bladder into the intestines where the bilirubin is changed into a variety of forms. The most important ones are stercobilin, which is excreted in the feces, and urobilinogen, where is reabsorbed back into the blood then back to the liver where it is either re-excreted or return to blood for transport to kidneys, finally is excreted as a normal component of the urine (Fig. 2.9).

The bilirubin molecule consists of two rigid planar dipyrrole units joined by a methylene bridge at carbon 10 and can exist as three isomers, i.e., IIIα, IXα, and XIIIα, with IXα being the natural structure formed from heme catabolism (67). Moreover, within cells, bilirubin appears to be present primarily within membranes and at submicromolar concentrations. Bilirubin itself is a very water-insoluble substance at 37°C and pH 8 (69). In extracellular fluids the bilirubin pigment present at 15 µM and predominantly bound to albumin, rendering the
otherwise highly lipophilic pigment water-soluble. Bilirubin is considered a strong reducing agent and a potential physiological antioxidant (3).

**2.4.2. Physiological protection role of bilirubin**

Several studies observed different circulation forms of bilirubin, which are acting powerful antioxidants: free bilirubin, albumin-bound bilirubin, conjugated bilirubin (free bilirubin is conjugated to either glucuronic acid or sulfate), and unconjugated bilirubin. All are effective as scavengers to peroxyl radicals and have the ability to protect human LDL against peroxidation (60). When it is bound to albumin the pigment will protect the cells against oxidative damage (70). In addition, bilirubin and more especially albumin-bound bilirubin are found to be cytoprotective to human erythrocytes and human myocytes during cells exposed to oxyradicals (9) (fig. 2.10).

The main role of bilirubin antioxidant action is via ROS process, the ROS of biliverdin and bilirubin generation are both potent scavengers of peroxyl radicals (71). The free and albumin-bound bilirubin are able to reduce O· and inhibit plasma LDL lipid peroxidation (9). Both biliverdin and bilirubin possess antioxidant activities and most of the actions are attributed to bilirubin via H-donation to an incipient radical, such as a lipid peroxyl radical (LOO·), to form lipid hydroperoxide (LOOH) and bilirubin radical (72).

These initial ROS (·O₂ and H₂O₂) are not only detrimental but also signal transduction molecules involved in several signaling cascades hydroxyl radicals which is easily react with cellular macromolecules, including DNA, proteins and lipids. In addition, both ·O₂ and H₂O₂ can interact with NO and form highly reactive peroxynitrite (ONOO⁻) (72).

![Fig. 2.10.: Reactive Oxygen Species (72)](image-url)
Therefore, the oxidation of bilirubin by ROS produces conversion of bilirubin biliverdin, as a bilirubin precursor in heme degradation and recycling it to bilirubin by *biliverdin reductase* in mammals (72). This recycling between bilirubin and biliverdin one of the explanations for bilirubin powerful antioxidant effects in ROS cycle (72).

To date, few experimental studies have attempted to provide direct evidence for bilirubin acting as an important antioxidant in vivo (73).

Moreover, Infants with disorders that involve oxygen radical-mediated injury, such as necrotizing enterocolitis, bronchopulmonary dysplasia, intraventricular hemorrhage, and retinopathy of prematurity, display lower circulating bilirubin than healthy controls (74). Likewise, a direct correlation was found between serum bilirubin concentrations and total antioxidant status in premature neonates (75).

Molecular biology field have allowed the expression manipulate of a particular gene-using gene targeting (76). The result of created HO-1 deficient mice is an oxidation of macromolecules and tissue injury arose spontaneously, so this evidence supporting the views about HO-1 that normally plays an antioxidant role (77).

Antioxidant activity and cardioprotective potential may be attributable to any of the bilirubin forms, including free unconjugated bilirubin, protein-bound unconjugated bilirubin, delta bilirubin, or mono-/diconjugated bilirubin (78). Moreover, the predominant circulatory form of bilirubin is the unconjugated and albumin-bound form. Nevertheless, it is not known whether conditions that modify the relative proportions of this form of bilirubin in the blood. The albumin-bound bilirubin was converted to biliverdin on oxidation by quenching 2 mol of peroxy radicals for each mole of bilirubin consumed (19).

Recent evidence has been indicated that biliverdin and bilirubin are synthesized as byproducts of the enzymatic reaction catalyzed by HO, which serve as key mediators that maintain the integrity of the physiological function of organs (72).
For many years, the bile pigment bilirubin was considered in high concentration as toxic waste product, which is the most abundant endogenous antioxidant and accounts for the majority of the antioxidant activity in human serum (72).

Generally, it was accepted that the oxidative reactions are involved in the pathophysiology of disease processes. High-normal plasma level of bilirubin was reported to be inversely related to atherogenic risk and to provide protection against endothelial damage. Risk reduction by bilirubin was comparable to that of HDL by CAD development retarding mechanism (80, 81). In addition, acts as a non-enzymatic scavenger involved in the antioxidant defense, which was observed in higher concentrations in the lungs of smokers than non smokers, suggesting up-regulation in these circumstances (8). Serum bilirubin levels correlate with a reduced risk of IHD (72). Therefore, the elevation of serum bilirubin levels have been consistently reported to protect from CAD in several studies (81). Hence, bilirubin production is involved in antioxidant defense mechanisms and that higher bilirubin concentrations are associated with a lower incidence of oxygen radical-mediated injury (76, 77, 84).

The oxidative stress was found to cause depletion of endogenous antioxidants, including bilirubin, in human plasma and to increase production of lipid hydroperoxides (82). In addition, other study has shown that the increase in serum bilirubin concentration (but still within the normal range) are associated with a significant and marked reduction in CAD risks (77).

Moreover, the relation between bilirubin and CAD was fully described. A U-shaped relationship between circulating bilirubin concentrations and cardiovascular risk was observed (83), leading to the conclusion that low concentrations of serum bilirubin are associated with increased risk of IHD (84). In addition, the relation of bilirubin with CAD risk factors was described by other investigators who found that plasma bilirubin correlated inversely with several known risk factors for CAD, such as smoking, LDL-cholesterol, DM, and obesity, and correlated directly with the protective factor HDL-cholesterol (42).
2.4.3. Other product related to bilirubin production

The process of removing damaged Hb, oxidized Hb or catabolized Hb by converting those into other products considered an important step in body physiological system to reutilizing and providing a cytoprotective mechanism. Therefore, the cytoprotective mechanisms are crucial for the defense of cells, tissues, and organs against noxious external and internal stressors. Heme oxygenase-1 functions by catabolizing heme to biliverdin, iron, and CO, these byproducts of heme degradation are believed to be effector molecules underlying the potent cytoprotection observed with the HO system (Fig. 2.11) (87, 88).

For many years bilirubin is known as a catabolic byproduct of heme oxygenase-1, is in physiological system the HO-1 induced by many stimulators (89, 90). Heme oxygenase-1 presents in both central and peripheral tissues (89), produces beneficial antioxidant metabolites, to prevent further cell damage, by conversion of pro-oxidant heme into antioxidant bilirubin, and other reaction products (89). The byproducts of HO system inducible form, including iron, CO and biliverdin, have been shown to exert potent cellular protective effects by anti-oxidant actions against oxidative stress in various settings (90). Biliverdin is subsequently reduced to bilirubin by biliverdin reductase (8).

The classic example is the formation of a bruise can occur in every cell; which goes through different colors as it gradually heals: red heme to green biliverdin to yellow bilirubin.

![Diagram of bilirubin production](image)

Figure 2.11 The reaction of bilirubin production (91).
The major antioxidant effect of HO-1 is mediated by at least 4 pathways:

- Removal of pro-oxidant heme by degradation (92), generation bilirubin is a potent scavenger to hydroxyl radical.
- Coinduction of ferritin to sequester free iron.
- Reduce the generation of hydroxyl radicals (OH) (93).
- And the suppression of the prooxidant monocyte chemoattractant protein -1 (MCP-1) (94).

The adaptive response contributes to the maintenance of vascular tone and potency in atherosclerotic vessels (60).

### Chemical bilirubin production equation

| Heme + 3 AH(2) + 3 O(2) | <=> | biliverdin + Fe(2+) + CO + 3 A + 3 H(2)O |

In the chemical reaction, HO-1 cleaved heme ring with requiring oxygen and nicotinamide adenine dinucleotide phosphate (NADP) and converted it to biliverdin, with the concomitant release of iron and emission of CO in equimolar quantities (85), via using the molecular oxygen as oxidizing agent.

By heme catabolism, whereas the system has an absolute requirement for NADPH and molecular oxygen, 3 moles of oxygen are consumed per mole of bilirubin formed; 1.5 moles are used for the oxidation of the tetrapyrrole including the ar-methene carbon bridge, and 1.5 moles of oxygen are needed to oxidize the NADPH (95).

A total of 5 to 6 moles of NADPH were consumed per mole of bilirubin formed. Four moles of NADPH are probably the minimum required for the formation of bilirubin; the additional 1 to 2 moles of NADPH may represent heme stimulated NADPH consumption via non-bilirubin pathways (96).
2.4.3.1. Free heme

Heme proteins play an important role in many physiological processes including oxygen transport, mitochondrial respiration, and signal transduction (67). Heme protein could be derived by either endogenous or exogenous sources. The majority of heme is present in Hb, whereas other sources of heme proteins include myoglobin and other sources (67). Free heme exerts cytotoxic effects through formation of oxygen free radicals and lipid peroxidation (67). Free heme is highly lipophilic and will rapidly intercalate into the lipid membranes of adjacent cells, and activate vascular endothelial cells resulting in an upregulation of adhesion molecules (97).

The endogenous sources of heme in neurons and glia would be derived mainly from cytoplasmic heme proteins and mitochondrial cytochromes and would be involved in the normal turnover of the heme-containing proteins.

The exogenous sources of heme could be derived from the death of neighboring cells that would release their heme proteins or from heme derived from Hb. The removal of the pro-oxidant heme, in turn, the breakdown of heme to three products, has its own significance in essential cellular metabolism and contributes to the suppression of oxidative stress (98).

2.4.3.2. Carbon monoxide (CO)

Carbon monoxide is the second product of physiological Hb catabolic system to bilirubin. A new paradigm has been emerging which shows that CO is one of metabolites through heme degradation by HO, while clearly the cytoprotective in lower amounts and the beneficial function of CO as a signaling molecule that exerts significant cytoprotection via an anti-inflammatory, vasodilating, and anti-apoptotic properties (99). Also, CO functions as a smooth muscle-relaxing mediator via activation of the soluble Guanylyl Cyclase (sGC)/cGMP signaling pathway (92). In addition, investigations on the beneficial physiological effects of CO revealed that this molecule exerts vasodilatory effects through cGMP-dependent smooth muscle relaxation.
In contrast, CO will be toxic at higher concentrations at cells, it is of high concentration in cigarettes.

Similar to the well-established vasodilator NO, CO binds to the heme moiety of sGC, causing activation of cGMP and resultant vascular relaxation (67). Although the affinity of CO for sGC is equivalent to NO, the potency of NO-stimulated cGMP production is 30–100 times greater than that for CO (67). While both NOS and HO-1 are CO-induced in times of stress, and the majority of evidence suggests that HO may serve both to regulate and to continue the effects of NOS following the initial stress response. Nitric oxide has been demonstrated to induce HO-1 and subsequent production of CO (67), because NO is both a potent vasorelaxant and a potential free radical through the formation of peroxynitrite radicals, and the maintaining of the vasodilatory properties of the molecule by means of CO-stimulated cGMP production. Additional cGMP-mediated effects of CO include neurotransmission (67).

2.4.3.3. Heme iron (Fe^{2+})

Iron byproduct liberated from heme degradation process and can be utilizing in other processes of body system, whereas the catalyzing of Hb by HO-1 will be associated with elevation of cellular iron content (73).

The beneficial effect of iron molecule appear during the oxidation or reduction of ferric acid to ferrous, and the liberated free iron is an extremely prooxidative molecule primarily through its role in the Fenton reaction (67).

In contrast, no cytoprotective properties of free iron have been described. The induction of HO-1 has been linked to the upregulation of ferritin (67). Moreover the circulate Iron ions bound to plasma transferrin then accumulate within cells in ferritin form. Iron protoporphyrin (heme) and iron-sulfur clusters serves as enzyme cofactors (100), in addition the iron released through HO activity drives the synthesis of ferritin and that ferritin by virtue of its iron-binding capacity provides protection to endothelial cells against oxidative damages (101). Ferritin is a ubiquitously existing intracellular protein that is able to effectively sequester intracellular iron and, hence, limit its prooxidative capacity (67).
2.4.4. The relationship of bilirubin with CAD development

Schwertner and his colleagues were the first to observe the significant inverse correlation between total bilirubin plasma concentrations and the prevalence of CAD. This important finding indicated that the lower serum bilirubin concentration than normal is associated with the presence of IHD (11).

In another study, it was noted that the patients with early familial CAD have an average total serum bilirubin of CAD patients lower than healthy control subjects. Beside to these findings in a prospective study in middle-aged British men, low bilirubin was suggested as an independent risk factor for CAD, and an inverse correlation was demonstrated between bilirubin concentration and CAD morbidity (80).

Further supporting of the existence of this inverse correlation was came from genetic variation in bilirubin concentration, with individuals with early CAD displaying lower bilirubin than unaffected persons (102). Many studies referred to this variation due to HMOX1 gene promoter polymorphism. In Japanese patients an association between the HO-1 genotype and CAD with hyperlipidemia, D.M. and smoking (35). Also, an association between the HO-1 class short repeat of GT (S) allele and slightly elevated total bilirubin levels was found indicated that individuals carrying this allele might has higher levels of HO-1 and an increased production of the endogenous antioxidant bilirubin(35).

In another study, the author found that carriers of the class S allele have slightly higher HDL levels and lower serum triglyceride levels, whereas TC and LDL levels were not different. This effect might be explained by the anti-inflammatory effect of HO-1, and the author demonstrated that the ability of bilirubin at physiological concentration effectively prevent the oxidation of LDL lipids (70).

In collectively, all these findings seemed to be reasonable to speculate that increased HO-1 upregulation in patients with short (GT)n promoter repeat alleles may exert a protective effect against atherosclerotic lesion formation via enhanced release of the antioxidant bilirubin (103).
Chapter Three

MATERIALS AND METHODS

3.1 Study design
The present study is across sectional study.

3.2 Target population
A study sample of 94 CAD Palestinian patients was collected from both sexes who were administered to Cardiology Department of El-shefaa Hospital in Gaza.

3.3 Settings and place of work
The practical parts of this work were performed at the genetic engineering laboratory of the Islamic University of Gaza, El-shefaa laboratory and Khalid laboratory the private laboratory at Medical laboratory.

3.4 Ethical considerations
The approval letter for the present study was obtained from the Helsinki committee at the Palestinian Ministry of Health (MOH). In addition, all the subjects involved in the present study signed a formal consent form about their agreement to be involved in the present study. All parts of the present study were performed in accordance with the Helsinki Declaration of 1975.

3.5 Permissions
The permissions of the present study were obtained from the Faculty of Postgraduate studies, General manager of El-shefaa hospital and Cardiology Department of MOH.

3.6 Questionnaire
An informative questionnaire was designed including the most important risk factors include BMI, smoking, DM, hypertension, stress, physical activity and family history and the specific diagnosis according to administration of physicians, also include other personal information such as age, work, height and weight (Appendix-1).
3.7 Material

3.7.1 Reagents

<table>
<thead>
<tr>
<th>Used Reagents</th>
<th>Supplier</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Bilirubin (Total and Direct) Kit®</td>
<td>Biosystems</td>
<td></td>
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<tr>
<td>Triglyceride Kit®</td>
<td>Biosystems</td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol Kit®</td>
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<td>HDL precipitation Kit®</td>
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<td>Normal and Elevated control reagent®</td>
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3.7.2 Instruments

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<td>Micropipette 10---1000 μL</td>
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<td>Water bath</td>
<td>G. engineering</td>
<td>PSELECTA, Spain</td>
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<td>El-shefaa central lab.</td>
<td>ATI LINI CAM- UNICAM8675 visible spectrophotometer</td>
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<tr>
<td>Spectrophotometer</td>
<td>El-shefaa central lab. and Khalid Lab.</td>
<td>Biosystems BTS-310 photometer</td>
</tr>
</tbody>
</table>

3.8. Collection of samples

- Samples collection began during the period of 1/6/2008 to 16/8/2008, at the Cardiology Department of El-shefaa Hospital. With taking into consideration safety rules and quality assurance guidelines, 5 ml venous blood were collected in plain vacationer tube by the researcher and nurse.

- About 94 fresh blood samples of fasted at least 12 hour CAD patients were collected into serum tubes and patients were face to face interviewed to fill in a questionnaire.

- Blood sample were collected incubated is left to stand for 30 minute at room temperature, centrifuged and serum was separated into new test tubes.
3.9 Chemical analysis:

3.9.1 Bilirubin measurements

Biosystems Bilirubin DIAZOTIZED SULFANILIC kit, Bilirubin (Total and Direct – Cod-11555 (500+500ml) kit number was used.

○ Reagent Preparation

*Working Reagent:* The working reagents were prepared by transferring the contents of one Reagent B vial into a Reagent AT bottle for total bilirubin determination, or into Reagent AD bottle for direct bilirubin determination. The reagents were mixed thoroughly. Another alternative methods, some tests were done by mixing 1 ml Reagent B + 4 ml Reagent AT or AD.

The working reagent was stable for 20 days at 2-8°C after preparation.

The samples which was used a fresh serum sample, and were collected by standard procedure.

○ Procedure for total bilirubin:

1. Depending on standard kit procedure, the reagents, serum samples and standard were added into the label test tubes as the following table.

<table>
<thead>
<tr>
<th>Chemicals Used</th>
<th>1st test tube</th>
<th>2nd test tube</th>
<th>3rd test tube</th>
<th>4th test tube</th>
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<tbody>
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<td>Reagent Blank</td>
<td>Blank</td>
<td>Sample</td>
<td>Standard</td>
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<td>Sample</td>
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<td>100 μl</td>
<td>---</td>
<td>100 μl</td>
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<td>Standard (S)</td>
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<td>---</td>
<td>100 μl</td>
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<td>Reagents (AT)</td>
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<td>Working Reagent</td>
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<td>---</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

2. Tubes were mixed thoroughly and incubated 2 minutes at room temperature.
3. The absorbance (A) of the standard and the sample were read at 540 nm against the distilled water.
4. The absorbance (A) of the standard and the sample were read at 540 nm against the reagent blank.
Procedure for direct bilirubin:
1. The pure serum of blood samples was separated by centrifugation and finished the reagent preparation. Depending on standard kit procedure, the reagents, serum samples and standard were added into the labeled test tubes as the following table.

<table>
<thead>
<tr>
<th>Chemicals Used</th>
<th>1st test tube</th>
<th>2nd test tube</th>
<th>3rd test tube</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blank</td>
<td>Blank</td>
<td>Sample</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>100 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td></td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>Reagents (AD)</td>
<td></td>
<td>1.0 ml</td>
<td></td>
</tr>
<tr>
<td>Working Reagent</td>
<td>1.0 ml</td>
<td></td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

2. Tubes were mixed thoroughly and incubated 5 minutes at 37°C.
3. The absorbance (A) of the standard and the sample was read at 540 nm against the distilled water.
4. The absorbance (A) of samples and of the standard was read at 540 nm against the reagent blank.

○ Calculation:
The bilirubin concentration in the samples was calculated by using general formula:

\[
\frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{standard}}} \times C_{\text{standard}} = C_{\text{sample}}
\]

- The calculations of direct bilirubin used the obtained absorbance value for standard in the total bilirubin procedure when 1cm cuvette was used for reading, the following factor was used:

\[(A_{\text{sample}} - A_{\text{sample blank}}) \times 7,74 = C_{\text{sample}} \text{ (mg/dl)}\]

- The automatic spectrophotometer was used to calculating directly the results.
3.9.2 Lipid profile measurements

A) Triglycerides

Biosystems Triglycerides – GLYCEROL PHOSPHATE OXIDASE/PEROXIDASE kit, Cod-111529 (2X250 ml) was used

- Reagent Preparation: Reagent and Standard were provided ready to use.
- Sample: was used a fresh serum samples
- Procedure:

1. After the reagents were brought into room temperature, were used the reagent, samples and standard were added into labeled test tubes as the following table.

<table>
<thead>
<tr>
<th>Chemicals Used</th>
<th>1st test tube</th>
<th>2nd test tube</th>
<th>3rd test tube</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blank</td>
<td>Standard</td>
<td>Sample</td>
</tr>
<tr>
<td>Triglyceride Standard (S)</td>
<td>---</td>
<td>10 µl</td>
<td>---</td>
</tr>
<tr>
<td>Sample</td>
<td>---</td>
<td>---</td>
<td>10 µl</td>
</tr>
<tr>
<td>Reagent (A)</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

2. Tubes were mixed thoroughly and incubated 15 minutes at room temperature.

3. The absorbance (A) of the standard and the sample was read at 500 nm against the blank. The color is stable for at least 2 hours.

- Calculation:

Triglyceride concentration of the sample was calculated by using:

\[
\frac{A_{\text{sample}}}{A_{\text{standard}}} \times C_{\text{standard}} = C_{\text{sample}}
\]

- The automatic spectrophotometer was used to calculating directly the results.
B) Total - cholesterol

Biosystems Cholesterol – CHOLESTEROL OXIDASE/PEROXIDASE kit, Cod-11506 (1X500ml) was used.

- **Reagent Preparation:** Reagent and Standard were provided ready to use.
- **Sample:**
  - Was used fresh serum sample, and was collected by standard procedure.
- **Procedure:**
  1. After the reagents were brought into room temperature, were used the reagent, samples and standard were added into labeled test tubes as the following table.

<table>
<thead>
<tr>
<th>Chemicals Used</th>
<th>1st test tube</th>
<th>2nd test tube</th>
<th>3rd test tube</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blank</td>
<td>Standard</td>
<td>Sample</td>
</tr>
<tr>
<td>Triglyceride Standard (S)</td>
<td>---</td>
<td>10 μl</td>
<td>---</td>
</tr>
<tr>
<td>Sample</td>
<td>---</td>
<td>---</td>
<td>10 μl</td>
</tr>
<tr>
<td>Reagent (A)</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

3. Tubes were mixed thoroughly and incubated 10 minutes at room temperature.
4. The absorbance (A) of the standard and the sample was read at 500 nm against the blank. The color is stable for at least 2 hours.

- **Calculation:**
  Cholesterol concentration of the sample was calculated by using:

  \[
  C_{sample} = \frac{A_{sample}}{A_{standard}} \times C_{standard}
  \]

- The automatic spectrophotometer was used to calculating directly the results.
C) Cholesterol HDL

Biosystems Cholesterol HDL Precipitation kit – COD 11648, was used.

○ Procedure: According to manufacturer instructions was followed up these steps:

1. The reagents, samples and standard were added into labeled test tubes as the following table.

<table>
<thead>
<tr>
<th>Chemicals Used</th>
<th>test tube Sample-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>200 µl</td>
</tr>
<tr>
<td>Reagent (A)(Cholesterol HDL Precipitation)</td>
<td>0.5 ml</td>
</tr>
</tbody>
</table>

2. Tubes were mixed thoroughly and incubated 10 minutes at room temperature.
3. Tubes were centrifuged for 10 minute at 4000 round per minute was.
4. The supernatant was collected carefully.
5. After the reagents (Cholesterol kit) were brought into room temperature, were used the reagent, supernatant serum samples and HDL standard were added into labeled test tube as the following table.

<table>
<thead>
<tr>
<th>Chemicals Used</th>
<th>1st test tube Blank</th>
<th>2nd test tube Standard</th>
<th>3rd test tube Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol Standard (S)</td>
<td>100 µl</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Cholesterol HDL Standard (S)</td>
<td></td>
<td>100 µl</td>
<td>---</td>
</tr>
<tr>
<td>Sample</td>
<td>---</td>
<td>---</td>
<td>100 µl</td>
</tr>
<tr>
<td>Reagent (A)</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

7. Tubes were mixed thoroughly and incubated 30 minutes at room temperature.
8. The absorbance (A) of the standard and the sample was read at 500 nm against the blank. The color is stable for at least 2 hours.
9. The spectrophotometer Biosystems BTS-310 photometer was used to calculating directly the results.
D) Cholesterol LDL

LDL was estimated by using the Friedewald equation:

\[
\text{LDL cholesterol} = \text{total cholesterol} - \text{HDL} - (0.20 \times \text{triglycerides})
\]

E) HDL/LDL ratio

The HDL/LDL ratio gives indication about the ratio of cholesterol HDL level to cholesterol LDL level. This ratio is used to evaluate the risk of cardiovascular disease, which is determined by dividing LDL cholesterol into the HDL cholesterol. The result of HDL/LDL ratio would be 0.33 (104), moreover, the goal of HDL/LDL ratio is to keep the ratio above than 0.3, with the ideal HDL/LDL ratio being above 0.4 (104) (Appendix-3).

F) LDL/HDL ratio

The ratio of LDL and HDL cholesterol proportion is important in evaluating the risk of cardiovascular disease. Therefore, the ratio of HDL to LDL is a useful parameter and tool to estimate overall cardiovascular risk (104) (Appendix-3).

G) Total cholesterol/HDL ratio

This ratio is used to evaluate the risk of cardiovascular disease. This ratio uses the total cholesterol to HDL cholesterol level. Moreover, the healthy obtained ratio would be 4:1; moreover, the goal should be keep to the ratio below 5:1; while the optimum ratio is 3.5:1 (104) (Appendix-4).
3.9.3 Quality control for analyses

The control supplied from Labtrol Pathological Bovine source, Cod: 30900, Lot: 1818N.

The analysis and testing in laboratory were running with quality control reagent within run, therefore the obtained results of tests by the controls were used during laboratories testing, result of the samples control within normal range as shown in the following table.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Result of Normal control used</th>
<th>Confidence limits</th>
<th>Result of Abnormal control used</th>
<th>Confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>175±5</td>
<td>155 - 195</td>
<td>242±5</td>
<td>214 - 270</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>101±5</td>
<td>89 - 113</td>
<td>245±5</td>
<td>215 - 275</td>
</tr>
<tr>
<td>Total Bilirubin (mg/dl)</td>
<td>1.80±5</td>
<td>1.49 - 2.11</td>
<td>4.60±5</td>
<td>3.81-5.39</td>
</tr>
<tr>
<td>Direct Bilirubin (mg/dl)</td>
<td>1.05±5</td>
<td>0.83 - 1.17</td>
<td>2.06±5</td>
<td>1.71 – 2.41</td>
</tr>
</tbody>
</table>

3.10 Statistical analysis

All the data obtained from the questionnaire, lipid profile, and total with direct bilirubin measurements were entered in SPSS version 15 software. The following tests were applied:

- Frequency and distribution
- Student T-test
- Chi square test
4.1. Study Population Description

The sample size in present study was 94 CAD cases. The cases were diagnosed by specialist physician. Fasting CAD inpatient were tested biochemically for cholesterol, TG, LDL, total bilirubin, direct bilirubin, HDL to LDL ratio, LDL to HDL ratio and total Cholesterol to HDL ratio. Other risk factor were collected by using a questionnaire for age, hypertension, DM, physical activity, live stress, work stress, cigarette smoking and genetic related degree.

Among the CAD patients in this study there were 68 (72.3%) males and 26 (27.7%) were females (Fig. 4.1).

Fig. 4.1 Sex distribution among CAD patients
4.1.1. The distribution of age groups in CAD patients

As shown in Figure 4.2 the mean age of total CAD patients was 57.3±12.8 year. By using t-test analysis we found that there was no significant difference between the mean age of males (56±13.2 year) and that of females (60.5±11.1 year), \( P = 0.130 \).

The age of patients were divided into 3 groups: group1 the older age group (≥66year), group 2 the moderate age group (46-65year) and group 3 the younger age group (≤45year). It was found that the older age group contains 21 CAD patients 22.3% (17.6% males and 34.6% females), the moderate age group contains 55 CAD patients 58.5% (60.3% males and 53.8% females) which is the higher distribution group and the lower age group contains 18 CAD patients19.1% (22.1% males and 11.6% females). By using chi square analysis to find the relation between patients, age and CAD occurrence risks, it was found that CAD patients who are at higher risk are the middle age patients (\( P < 0.001 \)) (figure 4.3).
4.1.2. The distribution of BMI in CAD patients

As shown in figure 4.4 the mean BMI of total CAD patients was 28.7±6.0 kg/m². By using t-test analysis we found that there was a significant difference between mean BMI of males (27.4±4.0 kg/m²) and that of females (31.9±8.8 kg/m²), P = 0.001.

![Fig. 4.4 The average of BMI in CAD patients](image)

The patients were divided into 3 groups: group1 the overweight group, group 2 the obese group and group 3 the normal weight group. It was found that the overweight group contains 29 CAD patients 31.0% (25% males and 46.2% females), the obese group contains 31 CAD patients 33.0% (33.8% males and 30.8% females) and the normal weight group contains 34 CAD patients 36.0% (41.2% of male and 23.1% of female). By using chi square analysis to find the relation between patients BMI and CAD occurrence risks, it was found that CAD patients who are at higher risk of overweight are female patients (P<0.001) and that CAD patients who are at higher risk of normal weight are male patients (P = 0.003). On the other hand there was no relation between male and female in obese group (P > 0.05) (figure 4.5).

![Fig. 4.5 Distribution of BMI in CAD patients](image)
4.1.3. The distribution of cholesterol in CAD patients

As shown in figure 4.6 the mean serum cholesterol of total CAD patients was (167.3±62.2) mg/dl. By using t-test analysis we found that there was no significant difference in mean cholesterol of male (166.0±57.7) mg/dl and the mean cholesterol of female (170.8±73.9) mg/dl ($P = 0.738$).

![Fig. 4.6 The average of total cholesterol in CAD patients](image)

In the present study the patients were divided into 3 groups based on their cholesterol levels: group 1 the high level group, group 2 the borderline level group and group 3 the normal level group. It was found that the high cholesterol level group contains 16 CAD patients 17% (19.1% males and 11.5% females), the borderline level group (under risk cases) contains 7 CAD patients 7.4% (7.4% males and 7.7% females) and the normal level group contains 71 CAD patients 75% (73.5% males and 80.8% females). By using chi square analysis to find the relation between patients serum cholesterol and CAD occurrence, risks it was found that CAD patients who are the higher risk at high level are male patients ($P < 0.008$), while there was no relation between male and female in other groups ($P > 0.05$) (Fig. 4.7).

![Fig. 4.7 The distribution of total cholesterol in CAD patients](image)
4.1.4. The distribution of serum triglyceride level in CAD patients

As shown in figure 4.8 the mean serum triglyceride of total CAD patients was (163.7±82.5) mg/dl. By using t-test analysis we found that there was no significant difference between the mean triglyceride of males (170.4±85.2 mg/dl) that of females (146.3±73.7 mg/dl), \( P = 0.207 \).

![Fig. 4.8 The average of serum triglyceride level in CAD patients](image)

In the present study there were divided the patients into 3 groups based on their serum triglyceride levels: group1 the high level group, group 2 the borderline high level group and group 3 the normal level group. It was found that the high level group contains 22 CAD patients 23.4% (26.5% males and 15.4% females), the borderline level group (under risk cases) contains 17 CAD patients 18.1% (20.6% males and 11.5% females) and the normal level group contains 55 CAD patients 58.5% (52.9% males and 73.1% females). By using chi square analysis to find the relation between patients serum triglyceride and CAD occurrence risks it was found that CAD patients who are the higher risk at high level and borderline level are male patients (\( P < 0.05 \)) (figure 4.9).

![Fig. 4.9 The distribution of serum triglyceride level in CAD patients](image)
4.1.5. The distribution of serum LDL level in CAD patients

As shown in Figure 4.10 the mean serum LDL of total CAD patients was (99.7±59.8 mg/dl). By using t-test analysis we found that there was no significant difference between the mean LDL of males (96.6±51.5 mg/dl) and that of females (107.9±78.4 mg/dl), $P = 0.415$.

In the present study the patients were divided into 2 groups based on their serum LDL levels group 1 the high level (undesirable) group and group 2 the normal level group. It was found that the high serum LDL level group contains 18 CAD patients 19.1% (20.6% males and 15.4% females) and the normal level group contains 76 CAD patients 80.9% (79.5% males and 84.6% females). By using chi square analysis to find the relation between patients serum LDL level and CAD occurrence risks it was found no relation between males and females in the two groups ($P > 0.05$) (figure 4.11).
4.1.6. The distribution of serum HDL level in CAD patients

As shown in figure 4.12 the mean serum HDL of total CAD patients was (34.8±12.5 mg/dl). By using t-test analysis we found that there was no significant difference between the mean HDL of males (35.3±13 mg/dl) and that of females (33.6±11.2 mg/dl), \( P = 0.569 \).

In the present study patients were divided into 3 groups based on their serum HDL levels group 1 the very high level group, group 2 the high level and group 3 and group 3 the low level (undesirable) group. It was found that the high serum HDL level group contains 5 CAD patients 6.4% (8.80% males and 3.9% females), the high level group contains 20 CAD patients 21.3% (20.6% males and 19.2% females) and the normal contains 69 CAD patients 72.3% (70.6% males and 76.9% females). By using chi square analysis to find the relation between patients serum HDL and CAD occurrence risks, it was found no relation among males and females in the three groups (\( P > 0.05 \)) (figure 4.13).
4.1.7. The distribution of total cholesterol to HDL ratio in CAD patients

As shown in figure 4.14 the mean total cholesterol to HDL ratio of total CAD patients was (5.3±2.8). By using t-test analysis we found that there was no significant difference between the mean total cholesterol to HDL ratio of males (5.0±1.9) and that of females (5.9±4.3), \( P = 0.195 \).

![Fig. 4.14.: The distribution of total cholesterol to HDL ratio in CAD patients](image)

In the present study, patients were divided into 3 groups based on their cholesterol to HDL ratios: group 1 the high ratio, group 2 the safe ratio group and group 3 the ideal ratio group. It was found that the high total cholesterol to HDL ratio group contains 59 CAD patients 62.8% (61.8% males and 65.4% females), the safe borderline ratio group contains 13 CAD patients 13.8 % (14.7% males and 11.5% females) and the normal ratio group contains 22 CAD patients 23% (23.5% males and 23.1% females). By using chi square analysis to find the relation between patients total cholesterol to HDL ratio and CAD occurrence risks, it was found that CAD patients who are at higher risk of total cholesterol to HDL ratio are high risk patients (\( P < 0.05 \)), while there was no relation between males and females in the three groups (\( P\text{-value} > 0.05 \)) (figure 4.15).

![Fig. 4.15: The distribution of cholesterol to HDL ratio in CAD patients](image)
4.1.8. The distribution of HDL to LDL ratio in CAD patients

As shown in figure 4.16 the mean HDL to LDL ratio of total CAD patients was (0.5±0.4). By using t-test analysis we found that there was no significant difference between the mean HDL to LDL ratio of males (0.5±0.4) and that of females (0.4±0.3), \( P = 0.541 \).

![Fig. 4.16 The average of HDL to LDL ratio in CAD patients](image)

In the present study, the patients were divided into 5 groups based on their HDL to LDL ratios: group 1 the high risk ratio, group 2 the moderate risk ratio group, group 3 the average risk ratio group, group 4 the low ratio group and group 5 the normal ratio group. It was found that the high risk group contains 1 CAD patients 1.1% (0.0% males 3.8% and females), the moderate risk group contains 2 CAD patients 2.1% (1.5% males and 3.8% females), the average risk group contains 16 CAD patients 17% (16.2% males and 19.2% females), the low risk group contains 19 CAD patients 20.2% (20.6% males and 19.2% females) and the normal level group contains 56 CAD patients 59.6% (61.8% males and 53.8% females). By using chi square analysis to find the relation between patients HDL to LDL ratio and CAD occurrence risks, it was found no relation among the five groups (\( P > 0.05 \)) or between males and females in the five groups (\( P = 0.541 \)) (figure 4.17).

![Fig. 4.17 The distribution of HDL to LDL ratio in CAD patients](image)
4.1.9. The distribution of LDL to HDL ratio in CAD patients:

As shown in figure 4.18 the mean LDL to HDL ratio of total CAD patients was (3.2±2.6). By using t-test analysis, we found that there was no significant difference between the mean HDL to LDL ratio of males (2.9±1.7) and that of females (3.9±4.2) \( P > 0.05 \).

In the present study the patients were divided into 5 groups based on their LDL to HDL ratios: group 1 the high risk ratio group, group 2 the moderate risk ratio group, group 3 the average risk ratio group, group 4 the low ratio group and group 5 the normal ratio group. It was found that the high risk ratio group contains 1 CAD patients 1.1% (4.0% males and 0.0% females), the moderate risk group contains 2 CAD patients 2.2% (4.1% males and 1.4% females), the average risk group contains 17 CAD patients 17.0% (20.0% males and 12.0% females), the low risk group contains 18 CAD patients 19.1% (25.0% males and 17% females) and the normal level group contains 57 CAD patients 60.7% (46.8% males and 69.7% females). By using chi square analysis to find the relation between patients LDL to HDL ratio and CAD occurrence risks, it was found no relation between males and females in the five groups (\( P > 0.05 \)) (figure 4.19).
4.1.10. The distribution of serum bilirubin concentration in CAD patients

As shown in figure 4.20 the mean serum total bilirubin concentration of total CAD patients was (0.9±1.2 mg/dl). By using t-test analysis we found that there was no significant difference between the mean serum total bilirubin concentration of males (1.0±1.4 mg/dl) and that of females (0.6±0.11 mg/dl), P > 0.147.

![Fig. 4.20 The average of serum total bilirubin concentration in CAD patients](image)

The patients in the present study were divided into 2 groups based on their serum total bilirubin concentrations: group 1 the high concentration group (>1.0 mg/dl), group 2 the low or normal concentration group (≤1.0 mg/dl). It was found that the high risk concentration group contains 8 CAD patients 8.8% (11.8% males and 0.0% females) and the normal level group contains 86 CAD patients 91.5% (88.2% males and 100.0% females). By using chi square analysis to find the relation between patients serum total bilirubin concentration and CAD occurrence risks, it was found no relation between males and females in the two groups (P > 0.05) (figure 4.21).

![Fig. 4.21 The distribution of serum total bilirubin concentration in CAD patients](image)
As shown in figure 4.22 the mean serum direct bilirubin concentration of total CAD patients was (0.5±0.8 mg/dl). By using t-test analysis we found that there was no significant difference between the mean serum total bilirubin concentration of male (0.5±0.9 mg/dl) and the mean of female (0.3±0.4 mg/dl), \( P = 0.243 \).

In the present study the patients were divided into 2 groups based on their serum direct bilirubin concentrations: group 1 the high concentration group (>0.2 mg/dl), group 2 the low or normal concentration group (≤0.2 mg/dl). It was found that the high risk concentration group contains 15 CAD patients 16% (17.6% males and 11.5% females) and the normal level group contains 79 CAD patients 84% (82.4% males and 88.5% females). By using chi square analysis to find the relation between patients serum total bilirubin concentration and CAD occurrence risks, it was found no relation between two groups or between males and females in the two groups (\( P = 0.475 \)) (figure 4.23).
4.1.11. The distribution of diabetic as risk factor in CAD patients

In the present study it was observed that the diabetic contains 36 CAD patients 38.3% (33.8% males and 50.0% females) and non diabetic 58 CAD patients 61.7% (66.2% males and 50.0% females). By using chi square analysis to find the relation between diabetic patients and CAD occurrence risks, it was found who are at higher risk of DM are diabetic female patients in CAD patients (P = 0.02) (figure 4.24).

![Fig. 4.24 The distribution of diabetes in CAD patients](image)

4.1.12. The distribution of hypertension as risk factor in CAD patients

In the present study, it was observed that the hypertensive contains 34 CAD patients 36.2% (35.3% males and 38.5% females) and normotensive 60 CAD patients 63.8% (64.7% males and 61.5% females). By using chi square analysis to find the relation between hypertensive patients and CAD occurrence risks, it was found no relation between males and females in the two groups with regard to the prevalence of CAD (P =0.723) (figure 4.25).

![Fig. 4.1.12.: Distribution of hypertension in CAD patients](image)
4.1.13. The distribution of physical activity groups as risk factor in CAD patients

In the present study the patients were divided into 3 groups based on their physical activity levels: group 1 the heavy physical activity group, group 2 the moderate activity group and group 3 the sedentary to light activity or non active group. It was observed that the heavy physical activity group contains 18 CAD patients 19.1% (24.0% males and 8.0% females), the moderate activity group contains 25 CAD patients 26.6% (29.4% males and 19.0% females) and the sedentary to light or inactive group contains 51 CAD patients 54.3% (47.1% males and 73.0% females). By using chi square analysis to find the relation between physical activity and CAD occurrence risks, it was found that CAD patients who are at higher risk of CAD are sedentary to light activity patients \( (P=0.001) \). In addition CAD patients who are at higher risk of CAD are females of sedentary to light activity patients \( (P=0.001) \) (figure 4.26).

![Fig. 4.26 Distribution of physical activity groups in CAD patients](image-url)
4.1.14. The distribution of cigarette smoking as risk factor in CAD patients

In the present study it was observed that smokers contain 34 CAD patients 44.7% (60.3% males and 3.8% females) and the non smokers contain 60 CAD patients 55.3% (3.8% males and 96.2% females). By using chi square analysis to find the relation between smoking and CAD occurrence risks, it was found no relation between two groups regarding (P =0.302) (figure 4.27).

![Distribution of smoker in CAD patients](image1)

**Fig. 4.1.14.: the distribution of smoker in CAD patients**

4.1.15. The distribution of life stress as risk factor in CAD patients

In the present study, it was observed that the life stress contains 29 CAD patients 30.9% (26.5% males and 42.3% females) and the non stress CAD contains 65 patients 69.1% (73.5% males and 57.7% females). By using chi square analysis to find the relation between life stress patients and CAD occurrence risks, it was found that CAD patients who are at higher risk of life stress are female patients (P =0.001) (figure 4.28).

![Distribution of life stress in CAD patients](image2)

**Fig. 4.28 Distribution of life stress in CAD patients**
4.1.16. The distribution of worker as risk factor in CAD patients

In the present study it was observed that the worker contains 56 CAD patients 59.6% (80.9% males and 3.8% females) and non worker 38 CAD patients 40.4% (19.1% of male and 96.2% of female). By using chi square analysis to find the relation between worker patients and CAD occurrence risks, it was found that male workers are higher than male non workers ($P = 0.03$) and that male workers are higher than female counterparts ($P = 0.001$) (figure 4.29).

![Fig. 4.29 Distribution of worker in CAD patients](image)

4.1.17. The distribution of family history as risk factor in CAD patients

In the present study the patients were divided into 3 groups based on their family history: group 1 first related genetic degree, group 2 second related genetic degree and group 3 non related genetic degree. It was observed that the first related contains 27 CAD patients 28.7% (32.4% males and 19% females) and, second related 3 CAD patients 3.2% (3.9% males and 4% females) and non related 64 CAD patients 68.1% (64.7% males and 76.9% females) (figure 4.30).

![Fig. 4.30 Distribution of family history in CAD patients](image)
In recent decades, the CAD is considered the major silent killer disease and the life threatened disease in different countries of the world. For best CAD prevention or retardation, the risk factors associated with CAD progression should be estimated. Therefore, in our study we tried to determine the association of risk factors associated with CAD progression of CAD patients in Gaza. According to our knowledge, no previous data were available about CAD risk factors in Gaza.

In the current study the reference values for optimal and high levels of serum lipids profile were based on the world studies as follows; the National Cholesterol Education Program (NCEP) detected the optimal serum TC level $< 200 \text{ mg/dL}$, borderline cholesterol level $200–239 \text{ mg/dL}$ and high cholesterol level $\geq 240 \text{ mg/dL}$. For serum TG level the optimal TG level $< 160 \text{ mg/dL}$, borderline TG high level $160–199 \text{ mg/dL}$ and high TG level $> 199 \text{ mg/dL}$. Moreover, the optimal serum HDL-C level $> 40 \text{ mg/dL}$ for both sexes, and for serum LDL optimal level was $< 100 \text{ mg/dL}$. These same values were used in regional studies such as; at Jordan (54), Islamic Republic of Iran (Qazvin) (2), Islamic Republic of Iran (Tehran) (1) and the epidemiological study of Arab women at Jerusalem of Palestine and Jewish women of Israel (105).

The risk factor elevation in under risk population may not be founded elevated in all patients, nearly 50% of cases of coronary events occur in the absence of the known classic CAD risk factors such as family history, dyslipidaemia, hypertension, smoking and DM. Many factors share to occur the CAD (2).

In this study, it was founded that the sex risk factor distribution of males and females was (72.3%: 27.7%). Similar result was observed (78.3%: 21.7%) in Jordanian study (54), but not similar in Qazvin population (50.2%: 49.8%) (2) and (41%: 59%) in Tehran studies (1).

In the current study the mean age of CAD patients was (57.27±12.78 year) similar to that in Tehran study (54±12 year). The mean age of female patients was (60±11 year) that is near to Arab and Jewish women study (68±9 and 63±9
Our study agreed with the result of Qazvin study. It was reported that the incidence of CAD after 40 years of age is 40% for men and 32% for women (2).

Also, it was observed that in the different age groups the most CAD patients was categorized in the middle group (45-66 year) to be 58.5% among three groups, while on other study conducted in Islamic Republic of Iran (Isfahan) showed that the overall prevalence of CAD was categorized in age groups (30–79 year) to be 19.4% (1). In this study, there was significant differences between males and females of each group (P <0.001). In Tehran study reported that the prevalence of probable CAD patient sexes was different between the age groups between males and females of each group and present a significant differences (1). Also in other regional studies there was observed a difference between age groups of both sex. The comparison of age groups of our study with other studies are difficult, because there were no limited age groups in the most studies, as referred in the Arabic and Jewish study (105). In addition, the incidence of CAD was considerably higher in Arabs of both genders than Jews. However, the presence of differences in risk factors between Palestinian women and Jewish women has showed that the Palestinian women are at higher risk for CAD (105).

In the current study, it was observed that the total mean of elevated BMI risk factor was (28.7±60 kg/m²), which is similar to (28.2±4.6 kg/m²) Tehran study (1), Qazvin study (27.5±90 kg/m²) (2) and Jordan study (27.5±50 kg/m²) (54). The BMI of CAD females was higher than males (P = 0.001), the distribution of total obese was 33.8% (33.8% males and 30.8% females) the distribution of females was lowered, this disagreed with Arabic and Jewish study which refers that the Palestinian women were obese more than Jewish women (105), and the female obese was higher than male as observed in Qazvin study (2). The total overweight CAD patients was 31.0% (25.0% males and 46.2% females), while in the overweight group the male percentage is significantly lower than female (P < 0.001). It was noticed that the main reasons for this result is related to the observation of Arabic and Jewish women study, which is reported that the certain psychosocial and behavioral factors are associated with BMI an
increased risk (105). Also this agreed with Qazvin and Tehran studies (1, 2). In which the author reported that the overweight and obesity are more common among their population women.

These demographic differences among the population of various studies emphasize the fact that each society has its own demographic characteristics and social behavior, which is reflected on the overall findings of each study.

5.1. Lipid profile

In our study, it was observed that the distribution of total patients of higher serum cholesterol cases (<200mg/dl) was 24.5% (26.5% of male and 29% of female) which is similar to 22% (19% males and 27% females) Jordanian study. The distribution of cholesterol of males was less than females, which agrees with the Jordanian study (54). The author referred that the cholesterol, TGs, and HDL were considered as independent CAD risk factors (33).

In our study, it was found that the average of cholesterol is the lowest of all the regional studies, while the percentage of cholesterol in males have a higher level of cholesterol than females (P =0.008). This agrees with Kuwaiti study, which found that the fasting level of hypertriglyceridemia more prevalent than hypercholesterolemia among Kuwaiti population and reported that the previous hypothesis of hypertriglyceridemia is an important risk factor for CAD in Kuwait (106). Our study does not agree with Tehran study (1) and Jordanian study (54), where borderline level (under risk cases) was 7.4% (7.4% males and 7.7% females). In addition, our result is supported by the speculation depends on estrogens hormone synthesized from cholesterol, which is lowering the cholesterol level, and will offer a protective effecting role to women body system (54).

The distribution of the TG (>150mg/dl) in our study as risk factor was 41.5% (47.1% males and 26.9% females) which was less than Qazvin study (53.5%) (2) and Jordan study (55%) (54). Moreover, in our study, it was found that the TG females are higher than TG males, which does not agree with (61.3% males and 52.4% females) Qazvin study (2). Moreover, the mean of serum TG 164 (170 in males and 146 mg/dl in females) is nearly similar to that (176 mg/dl) of
Jordan study (54), but the Jordanian study was reported that the mean of TG level of chronic CAD patients was significantly higher than non CAD patients. Also, it was observed that the male distribution is higher than female (54) which does not agree with our result. Our result found the distribution of males is significantly higher than females in triglycerides level of high and borderline groups (P <0.05), which agrees with Kuwaiti study, the author found that the hypertriglyceridemia was more prevalent in men than women which suggests that it may be the important cause of CAD death among Kuwaiti men. Also, he reported that the epidemiological, clinical and experimental studies confirmed that the hypertriglyceridemia predisposes individuals to thrombosis by increasing factor VII anticoagulant activity (106). And it increases plasminogen activator inhibitor, and may accelerate thrombotic processes (107). Therefore, the TG is considered important to predict the risk for the development of CAD (107). Only one published study, which found a strong and independent relationship between TGs, and CAD, while In contrast, most other studies have failed to demonstrate a strong independent association (33).

In our study, it was observed that the mean of total serum HDL was 34.8 mg/dL (35.3 males and 33.6 mg/dl females), which is nearly similar to the study conducted in the Islamic Republic of Iran study as it was referred in the Jordanian study. Also, it was reported that the level of HDL was 39.0 mg/dL in men and 46.5 mg/dL in women (54). As well, the author reported that the HDL mean is higher than the study of the Islamic Republic of Iran (34.5 mg/dL in men and 39.0 mg/dL in women) but lower than those in some European Mediterranean countries (46.2 in Italy, 46.6 in Spain and 51.1 mg/dL in France) (108).

The distribution of serum HDL (<40 mg/dl) as risk factor was low 72.3% (70.6% males and 76.9% females) which is higher than 54.0% (62.0% men and 47.0% women) in Qazvin study (2) and 45% (54.0% men and 27.0% women) in Jordan study (54) and Tehran study (1). In current study the mean of serum HDL females was higher than males, also higher than Jordanian study (54) and Tehran study (1), however, in Jordanian study the author referred to the mean of lowered HDL was significantly lower in females CAD patients (54), but it
doesn't agree with the Qazvin study. Moreover, in Qazvin study the author referred to the prevalence of CAD in African–American patients with low HDL level (2). In all that HDL groups (very high, high, and low) it was found that there is no significant difference between males and females ($P > 0.05$), which agrees with Tehran study (1), Qazvin study (2) and Jordan study (54). However, Jordanian study was reported that the differences between males and females distribution were related to the cut off value used in male and female >40mg/dl as our study has showed (54). As well, in Jordanian study the author reported that the only HDL cut-off value should not follow the NCEP reference values for optimal HDL of women, but they defined it as >40, rather than >50 mg/dl which leads to an underestimation of the number of women with low HDL level (54). Therefore, we should use the value of >45 mg/dl. The Lower HDL level and high TG level are recognized as independent coronary risk factors, and these may potentially play a more important role in the pathogenesis of atherosclerosis in this region of the world than hypercholesterolemia (54). While the relation of lower HDL level of CAD is not entirely known (106).

In the current study, it was found that the mean of serum LDL (99.7±59.8 mg/dl) is less than (135.4±38.7 mg/dl) Jordan study (54), while the mean of males is higher than females, which agreed with Jordanian study (54). In our study the distribution of serum LDL level ($\geq$161mg/dl) is 19.1% (20.6% males and 15.4% females), which is nearly similar to 21.1% (8.9 % males and 13.6% females) in Qazvin study. And in this study, it is observed that the male distribution is higher than female, which doesn't agreed with Qazvin study (2). Comparing Gaza population with the lifestyle and socioeconomic of Kuwait population in their study, it was observed that the level of LDL in healthy population was higher, and the author referred that It seems likely that diet may be an important contributory factor to high LDL mean and distribution as cholesterol mean, particularly the saturated fatty acids, which those with 12 to 16 carbon atoms and increases LDL-cholesterol. Also, the author referred that the Kuwaitis eat a lot of saturated fatty acids from meat and cholesterol from eggs, dairy products and sweets and this may partly explain their high serum total cholesterol concentrations (106). In addition, in another study was referred that the population of economically underdeveloped countries whose diet
contain low total calories, saturated fat, and cholesterol (34), but our population has different lifestyle and socioeconomic situation, therefore due to that because we notice the LDL mean and distribution was lowered. The LDL component plays the critical role in atherogenesis process particularly at the first step in CAD development by oxidation LDL process (23). Therefore, in the Jordanian study, it was referred that the true LDL level is 50-70mg/dl, which is precisely the normal range for individuals who live normal lifestyle and eat the diet for which they are genetically adapted, but he reported that the LDL level of 50-70 mg/dl seems excessively low by modern American standards. The author depended on the importance of LDL level in atherogenesis process and the differences in LDL levels in different populations; individuals living into the seventh and eighth decades of life and neonate LDL levels and in other creatures (66 and 109).

The mean of total cholesterol to HDL ratio as risk factor indicator was 5.3 (5.0 in males and 5.9 in females), which is lower than (6.7) Quebec Study (13), and as shown in our result the ratio of male is lower than female, while the male to female ratio is higher than Kuwaiti healthy population (3.7 in males and 3.4 in females) (106) but lower than (5.9 in males and 5.5 in females) (110). The increases or decreases of the total cholesterol to HDL ratio, HDL to LDL ratio and LDL to HDL ratio depends on the value of serum cholesterol, LDL and HDL levels. Moreover, each study has different cut off intervals, and few studies used these ratios. Therefore, it was difficult to compare these ratios with the ratios of other regional studies. The distribution of the high risk group of total cholesterol to HDL ratio (>4:1) was 62.8% (61.8% males and 65.4% females), and the safe borderline group was 13.8% (14.7% males and 11.5% females). The distribution of high risk group of total cholesterol to HDL ratio is significantly higher than safe and ideal groups (P <0.05), this significance was because the distribution value of serum HDL level was high in contrast, the value of cholesterol level was low. Therefore, the total cholesterol to HDL ratio is considered a good indicator for CAD prognosis and monitor. In the Quebec cardiovascular study the author referred that the total cholesterol to HDL ratio was a useful and simple index of IHD risk in men (13). The prevalence of total cholesterol to HDL ratio (>5) is considered an important warning risk factor
indicator for CAD, and the epidemiological studies have shown that with a combination of serum triglycerides >2 mmol/L (177 mg/dl) and HDL <1 mmol/L (38 mg/dl), will give us a strong indicator to a high risk of CAD (106).

The mean of LDL to HDL ratio as risk factor indicator was 3.2 (2.9 in males and 3.9 in females), which is nearly similar to (3.4) in South India study (111), but less than (3.7) in Tehran study (1), also the author of Tehran study reported high levels of LDL in their cases of CAD. It is possible that the presence of low HDL, even modest elevation of LDL will lead to subsequent elevation of LDL to HDL ratio and could contribute to CAD progression in patients and similar results have also been reported in India (1). Therefore, in the current study the ratio of LDL to HDL ratio mean was lower than the regional studies, due to the decrease of serum LDL level. In addition, the distribution of total high risk groups of LDL to HDL ratio (3.2) was 39.3% (53.1% males and 20.4% females), The elevation of LDL to HDL ratio depended on LDL and HDL level, also leads to the elevation of LDL to HDL and total cholesterol to HDL ratio and could contribute to atherogenesis in the population (111), and according to this author in Quebec study which suggested that a high LDL to HDL ratio combined with hypertriglyceremia, and will be associated with the highest CAD risk occurrence. In addition, the variation in the total cholesterol to HDL ratio may be associated with more substantial alterations in metabolic and indicates the prediction of IHD risk, and may be related to the insulin resistance syndrome (13), therefore and according to our results, the total cholesterol to HDL ratio is considered a good indicator to CAD estimation.

The mean of HDL to LDL ratio as a risk factor indicator was 0.5 (0.5 in males and 0.4 in females) which is nearly similar to 4.6 in Quebec Study (13). Moreover, the distribution of total high HDL to LDL ratio groups (<0.3) was 40.3% (61.4% males and 46.0% females), this ratio depends on serum HDL and LDL level. By comparison, this ratio with LDL to HDL ratio, we found that the HDL to LDL ratio and LDL to HDL ratio is not clearer indicator to CAD incidences.

In general, lipid profile is determined mainly by the quality and quantity of food whether it is fat-rich or low-fat diet. This affects the lipid blood levels. Each
society has its own food habits other risk factors should be taken into consideration as well. These facts may explain-in part, the differences between our study and other studies.

5.2. Total and direct serum bilirubin concentration

In the present study, we observed that the distribution of the normal or lowered group of serum total and direct bilirubin concentrations (≤1.0 and ≤0.2 mg/dl) were (91.5% and 88.2%), which agreed with Utah study (102) and other studies (20 and 33). In addition, our result was supported by many clinical studies, which include the study conducted in 1994 by Schwertner and his colleagues they were the first observers of the significant inverse correlation between total bilirubin plasma concentrations and the prevalence of CAD (11). Also, Stocker and his colleagues confirmed a strong inverse association between serum bilirubin and risk for early familial CAD (19). He was reported that the reduction in benefits of antioxidant bilirubin role may be consistent with current cigarette smokers, also he was the first demonstrator of the bilirubin antioxidant properties, where he referred that it may have a physiological significance. In fact, the serum total bilirubin is an independent cardiovascular risk factor such as cholesterol, TG and HDL (35). However, the serum bilirubin had an inverse relationship with CAD risk factors as serum cholesterol, cigarettes smoked/day, high systolic blood pressure, serum TG and fasting glucose (11).

Our study found no significant difference between male and female serum total bilirubin or serum direct bilirubin concentrations (P >0.05). The result of the serum bilirubin concentration in our study agrees with the observation of the study conducted in the United States population (112), where the author reported that differences in the distribution of lower group (≤=1mg/dl) of serum total bilirubin concentration was 91.1% and of higher group (>1.0 mg/dl) was 8.9%, which explains the concentrations of peripheral arterial disease (PAD) cases in one table, as well, it referred to PAD as an important manifestation of atherosclerosis and it was associated with significant morbidity (112).
5.3. Other associated CAD risk factors:-

The distribution of sedentary or nonphysical active as risk factor was 54.3 % (47.0% males and 73.0% females), females was much higher than males, and higher than (44.0% and 21.0%) Jewish and Palestinian women (105) and (16%) in Qazvin study (2). The distribution of sedentary or nonphysical activity is significantly higher than those with moderate and heavy activities (P =0.001). Moreover the distribution of sedentary physical activity among females was significantly higher than their male counterparts (P = 0.001), which agreed with Tehran study (1). Also, in Tehran study, the author referred that the women has lower levels of physical activity than men in general. Moreover, our result agreed with Arabic and Jewish study (105), where the author reported that the Arabic women has less physical activity exercise, and the higher physical active population is protected by exercise, more than physical light and moderate populations (105). Therefore, we suggested the same speculation in our population.

In our culture, females feel shy when they perform physical activity, for this reason the majority of females do not conduct activity at regular basis, in addition, many of the females in our society remain in their homes for long periods.

The distribution of diabetic patients as risk factor was between both sexes 38.3 % (33.8% males and 50.0% females) less than (51.0%) in Jordanian study (54) but more than (13.0%) in Qazvin study. In addition, the distribution of diabetic females was significantly higher than diabetic males (P = 0.02), which agreed with Qazvin study where the author referred that the sex distribution was observed in females more than males (2). However, female distribution is nearly similar to (61.0% and 46.0%) of Palestinian and Jewish women where the author reported that the Palestinian women have a greater diabetic distribution than Jewish ones (105).

The distribution of hypertensive patients as risk factor was 36.2 % (35.3% males and 38.5% females) much similar to 38.0% in Tehran study (1). In addition, our study found that the female distribution is higher than male, similar to Qazvin study (2), Female distribution was less than (73.0%) among Jewish
women and (65.0%) among Palestinian women (105). Our result is similar to Arabic and Jewish study (105). In addition, in Qazvin study the author referred that the CAD prevalence was high in African–American patients with hypertension in both sexes and all races (2).

The distribution of cigarettes smokers as risk factor was 44.7 % (60.3% males and 3.8% females), which is higher than (28.0%) in Jordanian study (54), (26.0%) in Jewish, (21.0%) in Palestinian women (105) and 16.1% (30.5 males and 1.9 females) in Qazvin study (2), but less than (88.0%) in Tehran study (1). However in our study no significant differences was found between smokers and non-smokers with regarded to the prevalence of CAD (P =0.302). Depending on other studies of non-smokers, we considered the persons reported regular smoking in the prior of 6 months as current smokers (39). Also, another study found an association between IHD and smoking, but in this study, there was no significant difference (113). Our result agreed with the result of Arabic and Jewish study, in which the author referred that the Arab women are less smoking but suffered more from passive smoking (105), and we observed the distribution of cigarettes smokers of CAD patients were higher than non-smokers in respect to any regional studies but less than some other global studies. The CAD was reported to be more prevalent in smokers than non-smokers in all races and both sexes (2). Moreover, epidemiologic studies strongly supported the assertion that cigarette smoking in both men and women increases the incidence of MI and fatal CAD, about one-hundredth of active smokers increase 80% risk of CAD (39). However, the cigarettes smoking increase TC, TG, LDL except HDL decreased (39).

The distribution of patients life stresses as risk factor was 30.9 % (26.5% males and 42.3% females) and the distribution of CAD females with life stresses was significantly higher than male patients (P = 0.01). This agreed with Arabic and Jewish study (105). However, we have suggested that the same condition is present in our population, especially the population of Gaza who are suffering from low socioeconomic and other conditions and this causes stress to population. Also, in Arabic and Jewish study, the author referred that the Palestinian women has less education and lower socioeconomic status than
Jewish women. In addition, the Palestinian women patients were younger and had more children than Jewish women (105), and the socioeconomic status particularly in women has consistently been associated with increased cardiovascular morbidity and mortality (105). The psychosocial stress and lifestyle factors are related to most of increased risk factors.

The distribution of worker was 59.6% (80.9% males and 3.8% females) and the distribution of males was much higher than females, due to the little number of old working women in our population. The percentage of worker CAD patients are significantly higher than non-worker CAD patients \( P = 0.03 \). Moreover, the percentage of male CAD workers are significantly higher than their female counterparts \( P = 0.001 \). This is because the distribution of women workers in our population was less than non-worker especially in older women. The stress of work plays a role in CAD development, the male is higher than female.

The distribution of family history of genetic related degree as inherited risk factor was 28.7% (32.4% males and 19.2% females) and second degree was 3.2% (3.9% males and 3.8% females). Female sex distribution was located between (44.0% and 12.0%) in Jewish and Palestinian women (105), the author reported that the prevalence of the family history was higher in the Jewish patients, and he referred that the premature CAD family history is an independent predictor of coronary risk in women, particularly among younger individuals with a family history of premature disease. The reliability of a self-reported family history of CAD or of risk factors for CAD has been questioned (105). In addition, in Arabic and Jewish study the author suggested that the additional contribution of family history to CAD risk estimation after inclusion of other traditional risk factors is relatively modest. He did not provide enough information about Palestinian population in this subject. In contrast, in the Jerusalem Lipid Research Clinic Study, the Jewish family history was an independent predictor among their population for CAD development (105).
6.1. Conclusions

- From our study we observed that the risk factors value and lipid profile levels are the important way for expectation of the CAD progressive in patients, which are must be taken in interest during the treatment and monitor of CAD patients under risk.

- In our study the HDL level of CAD patients was observed a more indicator suitable than other lipid profile levels. However the cutoff used in major previous studies for LDL>150mg/dl is less indicator than LDL>70 mg/dl for CAD diagnosis or prognosis CAD progression.

- Serum total and direct bilirubin concentrations in CAD patients showed strong inverse relation with CAD development, they play an important antioxidant role.

- Cigarette smoking is the most risk factor related to CAD incidence, which is the most of risk factor were distributed in CAD patients.

- Lowered physical activity was observed among CAD patients, and mainly in female patients.

- Stress is one of CAD risk factors which play a role for accelerating CAD, where as the females were at higher risk.
6.2. Recommendations

- According to our result the value of HDL is more positive diagnostic test to detect CAD occurrence, so should be interesting with patients HDL level during diagnosis, monitoring and treatment stages.

- Depending on results of our study, we recommended to replace the interval value of serum HDL of women from >40 to >45mg/dl.

- In our study LDL levels where shown to be less indicator, therefore we recommended to apply the LDL level new cut off >70mg/dl, particularly in CVD patients, Diabetic and patients under risk.

- We advancing to taken the bilirubin concentration in interesting in the treatment and evaluate the development of CAD and make another new studies to utilize the bilirubin for treatment.

- Also we recommended to apply the Framingham Heart Study in patients under risk (patients has high score risk factors) of our population for better monitor and treatment.

- Educational programs about smoking (passive smoking) and its bad role on whole body system, lowered physical activity especially in female population and life stresses are recommended.

- Further studies on CAD patients and normal population in the same field, are needed to estimate the baseline and differences of all risk factors in our population, for better diagnosis, treatment and monitor.
Chapter Seven

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APPENDIX

1. Questionnaire (appendix-1)

<table>
<thead>
<tr>
<th>Personal information :</th>
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<tbody>
<tr>
<td>Name :</td>
</tr>
<tr>
<td>Occupation:</td>
</tr>
<tr>
<td>Address :</td>
</tr>
<tr>
<td>Marital status:</td>
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<table>
<thead>
<tr>
<th>Question</th>
<th>Degree</th>
<th>key of Details</th>
</tr>
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<tbody>
<tr>
<td>- Are you diagnosed as having Hypertension?</td>
<td>1. Yes</td>
<td>2. No.</td>
</tr>
<tr>
<td>- Are you diagnosed as having Diabetes mellitus?</td>
<td>1. Yes</td>
<td>2. No.</td>
</tr>
<tr>
<td>- Are you diagnosed as having Hypercholesterolemia?</td>
<td>1. Yes</td>
<td>2. No.</td>
</tr>
<tr>
<td>- How old are you when you suffered of CAD?</td>
<td>At :-</td>
<td>4. 6.</td>
</tr>
<tr>
<td>- have any other defects?</td>
<td>Is :-</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Chemical analysis</th>
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<tbody>
<tr>
<td>Analysis</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>1. Total bilirubin</td>
</tr>
<tr>
<td>2. Direct bilirubin</td>
</tr>
<tr>
<td>3. Cholesterol</td>
</tr>
<tr>
<td>4. HDL-c</td>
</tr>
<tr>
<td>5. LDL</td>
</tr>
<tr>
<td>6. Triglyceride</td>
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</table>

Other Commends :
2. Bilirubin Guideline:
Bilirubin References values according to Biosystems kit.

<table>
<thead>
<tr>
<th>APPENDIX-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEST</td>
</tr>
<tr>
<td>TOTAL</td>
</tr>
<tr>
<td>DIRECT</td>
</tr>
</tbody>
</table>

3. Lipid Profile Guideline: NCEP Blood Lipid Guidelines and WHO, American Heart Association, NIH and Biosystems kit provide a set of lipid profile guidelines, as of 2003, these guidelines were:

**Note:**
- This information is relevant to triglyceride levels as tested after fasting 8 to 12 hours. Blood samples should be obtained after fasting.
- Triglyceride levels remain temporarily higher for a period after eating.

<table>
<thead>
<tr>
<th>Appendix-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dl)</td>
</tr>
<tr>
<td>&lt;200</td>
</tr>
<tr>
<td>200-239</td>
</tr>
<tr>
<td>&gt;=240</td>
</tr>
</tbody>
</table>

| Triglycerides (mg/dl) |
| <150 | Normal |
| 150-199 | Borderline High |
| 200-499 | High risk factor |
| >500 | Very High risk factor |

| HDL Cholesterol (mg/dl) |
| <40 | Low (undesirable) |
| >60 | High (desirable) |

| LDL Cholesterol (mg/dl) |
| <100 | Optimal |
| 100-129 | Near Optimal |
| 130-159 | Borderline High |
| 160-189 | High |
| > 190 | Very High |

*The National Cholesterol Education Program (May 16, 2001), Journal of the American Medical Association*

**According to Vitamin Research Products web site:**

<table>
<thead>
<tr>
<th>Appendix-3</th>
<th>Appendix-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk Level</td>
<td>LDL/HDL Ratio</td>
</tr>
<tr>
<td>Low risk (target goal)</td>
<td>3.3 - 4.4</td>
</tr>
<tr>
<td>Average risk</td>
<td>4.4 - 7.1</td>
</tr>
<tr>
<td>Moderate risk</td>
<td>7.1 - 11.0</td>
</tr>
<tr>
<td>High risk</td>
<td>11.0</td>
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