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Clinical Studies for Serum High Sensitivity C Reactive Protein, Oxidative Stress, Lipid Profile and Atherogenic Index in Patients with Acute Coronary Syndrome in Thi-Qar Governorate

A thesis

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Biochemistry

By

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بسم الله الرحمن الرحيم

رَبِّ اشْرَحْ لِي صَدْرِي ۞ وَيَسِّرْ لِي أَمْرِي ۞

وَاحْلُلْ عُقْدَةً مِنْ لِسَانِي ۞ يَفْقَهُوا قَوْلِي ۞

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CERTIFICATE

We certify that this thesis, entitled ***Clinical Studies for Serum High sensitivity C Reactive Protein, Oxidative Stress ,Lipid Profile and Atherogenic Index in Patients with Acute Coronary Syndrome in Thi-Qar Governorate***, was prepared by ***HADEEL RASHEED FARAJ*** under our supervision at the Department of Chemistry, College of Sciences , University of Thi-Qar (Iraq) in partial fulfillment of the requirements for the Master degree of science in chemistry.

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We, the examining committee, after reading this thesis "***Clinical Studies for Serum High sensitivity C reactive protein, Oxidative stress , Lipid profile and Atherogenic index in patients with Acute coronary syndrome in Thi-Qar Governorate***" and examining the student ***Hadeel Rasheed Faraj*** in its content, find that it is qualified for pursuing the degree of master of Science in Biochemistry with grade of **(Excellent)** on **(November 15th , 2012).**

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الاهداء

إلى الطاهرة المحدثة...إلى الصديقة المقدسة...سيدتي ومولاتي

فاطمة الزهراء...عليها السلام...

إلى صرح التضحية والوفاء ...وباباً لقضاء الحوائج...سيدتي ومولاتي

أم البنين...عليها السلام...

إلى من أحمل أسمه بكل إفتخار.. إلى من كلّت أنامله ليقدم لنا لحظة سعادة ..

إلى من حصد الأشواك عن دربي ليمهد لي طريق العلم....والدي العزيز

إلى ملاكي في الحياة .. إلى معنى الحنان والتفاني .. إلى سر الوجود ..إلى من كان دعائها سر نجاحي وحنانها بلسم جراحي .....والدتي الحبيبة

إلى من شاركني حضن الأم وبهم أستمد عزتي وإصراري .....أخوتي

بكل الحب.. إلى رفيق دربي إلى من سار معي نحو العلم..خطوة بخطوة .بذرناه معاً. وحصدناه معاً وسنبقى معاً. بإذن الله .جزاه الله خيراً ...زوجي الغالي محمد

إلى الوجه المفعم بالبراءة...وريحانة حياتي.....ولدي المصطفى

إلى الذين أحبتهم وأحبوني.. إلى من زرعوا التفاؤل في دربي وقدموا لي المساعدات .....عائلتي الثانية .أهل زوجي.

إلى صاحبة القلب الطيب والنوايا الصادقة..إلى من رافقتني في دروب الحياة.. إلى من تطلعت لنجاحي بنظرات الأمل.....خالتي أم سارة

إلى الأخوات اللواتي لم تلدهن أمي .. .. صديقاتي

أهديكم جميعا ثمار مسيرتي و جهدي راجيةً أن تقبلوه مني

هديل

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Contents Index

List of Abbreviations

4-AP	4-Aminophenazone
4-HNE	4-hydroxynonenal
A	Absorbance
ACS	Acute Coronary Syndrome
ADP	Adenosine di phosphate
AI	Atherogenic index
AMI	Acute Myocardial Infarction
AMP	Adenosine -5-mono phosphate
ATP	Adenosine tri phosphate
AP5A	Di adenosine 5 penta phosphate
CAD	Coronary Artery Diseases
CCU	Coronary Care Unit
CHD	Coronary Heart Diseases
CHE	Cholesterol esterase
CHOD	Cholesterol oxidase
CM	Chylomicrons
cm	Centimeter
Cp	Ceruloplasmin
CPK	Creatine phospho Kinase
CRP	C Reactive Protein

CVD	Coronary Vascular Diseases
dL	Deciliter
DM	Diabetes Mellitus
DNA	Deoxyribonucleic acid
D.W	Distilled Water
ECG	Electro Cardio Graphic
EDTA	Ethylen diamine tetra acetic acid
FER $_{HDL}$	Fractional esterification rate
FRs	Free Radicals
G-6-PDH	Glucose -6-phosphate dehydrogenase
GK	Glycerol kinase
gm	Gram
GPO	Glycerol-3-phosphate oxidase
GSH	Reduced Glutathione
GSSG	Oxidized Glutathione
H_2O_2	Hydrogen peroxide
HDL	High density lipoprotein
HK	Hexo Kinase
HOCl	Hypochlorous acid
hsCRP	High sensitivity C reactive protein
HTN	Hypertension
IDL	Intermediate density lipoprotein

IHD	Ischemic heart disease
IU/L	International Unit per Liter
KDa	Kilo Dalton
L	Liter
LCA	Lift coronary artery
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
LH	Lipid hydro perxides
LPO	Lipid peroxidation
MDA	Malondialdehyde
$MgCl_2$	Magnesium chloride
min	Minutes
µL	Microlitter
mL	Milliliter
NAD^+	Nicotine amide –adenine dinucleotide
$NADP^+$	Nicotinamide adinine dinucleotide phosphate (oxidized)
NaN_3	Sodium azide
nm	nanometer
nmol	nano mole
NQMI	Non-Q-wave myocardial infarction
NSTEMI	Non-ST segment elevation myocardial infarction
O_2	Oxygen

$^{\bullet}O_2^-$	Superoxide ion
OD	Optical density
$OH^{\bullet}$	Hydroxyl radical
WHO	World Health Organization
$ONOO^-$	Peroxy nitrite
PAP	4-Amino-antipyrine
POD	peroxidase
PPD	Para-phenylene diamine
PUFA	Poly Unsaturated Fatty Acid
QWMI	Q-wave myocardial infarction
r	Correlation coefficient
RCA	Right Coronary Artery
ROS	Reactive oxygen species
$RO^{\bullet}$	Alkoxy radical
$ROO^{\bullet}$	Peroxy radical
$ROOH^{\bullet}$	Organic hydroperoxide
rpm	Rotor per minute
SD	Standard deviation
SFBC	Societe Francaise de Biologie Clinique
SPSS	Statistical package social science
SOD	Superoxide dismutase

ST	ST segment of ECG tracing
TBARS	Thio barbituric acid reactive substance
TCA	Trichloro acetic acid
TCH	Total cholesterol
Tf	Transferrin
TG	Triglyceride
TIBC	Total Iron Binding Capacity
TS	Transferrin saturation
U/L	Unit per Liter
UA	Unstable Angina
UV	Ultra-Violate
VLDL	Very low density lipoprotein
WR	Working reagent

Summary

Ischemic heart diseases (IHD) is estimated to be the leading cause of mortality in the world and in high-income countries. The present study is designed to determine the levels of high sensitivity C reactive protein **(hsCRP)**, myocardial enzymes (creatine phospho kinase, lactate dehydrogenase) **(CPK,LDH)**, Oxidative stress (malondialdehyde) **(MDA),** (ceruloplasmin**,** transferrin**) (Cp,Tf)** , lipid profile (cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, very low density lipoprotein) **(TCH, TG, HDL, LDL, VLDL)**, and (atherogenic index) **(AI)** in patients with (Acute coronary syndrome(**ACS**)) and healthy individuals and asses the titer of hsCRP in two types of (**ACS**) (acute myocardial infarction **(AMI)** and unstable angina/NSTEMI **(UA)**) to predict its role in risk stratifications of patients with Acute coronary syndrome and healthy individuals.

The study includes **(170)** subjects which are divided into three groups: **(55)** patients of (AMI) [40 males and 15females] with age range (42—78). , **(45)** patients of (UA) [32 males and 13 females] with age range (39—80). and **(70)** supposed healthy subjects (control) [47 males and 23 females] with age range (35 −80). The study aims to shed a light on the possible correlation relationships between high sensitivity C reactive protein and each one of the studied parameters.

The results show that there is a significant increase in serum concentrations of each (hsCRP), (LDH), (MDA) ,(Cp), (TCH), (TG), (LDL), (VLDL), (AI) in all patients groups in comparison with control group ($P \leq 0.01$) and a non-significant increase in serum concentrations of

the mentioned parameters in group (AMI) in comparison with group (UA). Also there is a significant increase in concentrations of serum CPK-MB isoenzyme in all patients groups in comparison with(control) group ($P \leq 0.01$) and a significant elevation in the concentrations of serum CPK-MB isoenzyme in group (AMI) in comparison with group (UA) ($P \leq 0.01$).

Whereas there is a significant decrease ($P \leq 0.01$) in concentrations of serum (Tf , HDL)in all patients groups in comparison with (control) group and a non-significant decrease in serum concentrations of (Tf, HDL) in group (AMI) incomparison with group (UA) .

Also the study reveals a positive correlation between hsCRP and the levels of each (MDA, Cp, LDH, TCH, TG, LDL, VLDL, AI) and a negative correlation with the concentrations of (HDL and Tf)in patients with (AMI) and (UA).

While the correlation between hsCRP and CPK-MB isoenzyme is positive in patients with AMI and negative in patients with UA .

CHAPTER ONE

INTRODUCTION

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Ischemic heart disease (IHD) is estimated to be the leading cause of mortality in the world and in high-income countries, it is also the leading cause of premature mortality and disability[1]. Myocardial ischemia occurs as a result of diminished coronary blood flow, such reduction in blood flow can be partial or total, and is usually attributable to thrombus formation over an atheroma plaque, with occlusion of the coronary vascular lumen[2].

Some statistics pointed to recorded death rates from IHD vary widely across countries; age-standardized rates for males for the population aging over 30 range from more than 900 per100,000 in some Eastern European countries to 84 per 100,000 in Japan; for females the corresponding range is from more than 500 per 100,000 in some countries to less than 50 per 100,000 in France and Japan, understanding the huge variation in IHD mortality has been the focus of intense study in the last 20 years [3-8]. An example of the interest generated by the cross-national variation in IHD mortality rates is the so- called "French Paradox" - where France has a relatively high prevalence of the major risk factors for IHD such as Tobacco and poly unsaturated fatty acid intake but low reported IHD mortality rates [9- 11]. There is descriptive and analytical epidemiological study has been inspired by the large variation in IHD mortality rates across countries [12]. Not only has the cross-sectional pattern of IHD been an important stimulus to hypothesis formulation in this area but the recorded rise in many high income countries in IHD mortality rates in the 1950 and 1960 followed by declines in age-specific death rates has led to a vast body of research to understand the broader

determinants of IHD incidence, case-fatality rates and mortality rates [13-18].

Doubts have, however, been raised about the validity of cross-national comparisons. The term acute coronary syndromes is applied to the spectrum of three acute catastrophic manifestations of IHD: e.g. unstable angina / NSTEMI, acute myocardial infarction , and sudden cardiac death, all three cases result from acute changes in the morphology of atherosclerotic plaques[19,20].

Nasiriyah Heart Center recorded the number of patients with heart diseases for the year **2011** are **(10776)** and for the year **2012** are **(13276).**

With this increase in incidence in various types of diseases ,it is important to study the new prognosis parameters of these diseases.

1-1: Acute Myocardial Infarction (AMI):

The classic *World Health Organization (WHO)* criteria for the diagnosis of AMI require at least two of the following three elements to be present:

(1) A history of ischemic-type chest discomfort.

(2) Evolutionary changes on serially obtained electrocardiogram (ECG) tracings.

(3) A rise and fall of serum cardiac markers [21].

Approximately one million cases of perioperative cardiac complications, and 50,000 cases of AMI are diagnosed annually, with significant morbimortality and medical costs[22,23].

In a rare case, infarction may be due to coronary artery occlusion caused by coronary emboli, congenital abnormalities, coronary spasm, and wide

variety of systemic, particularly inflammation disease[24].

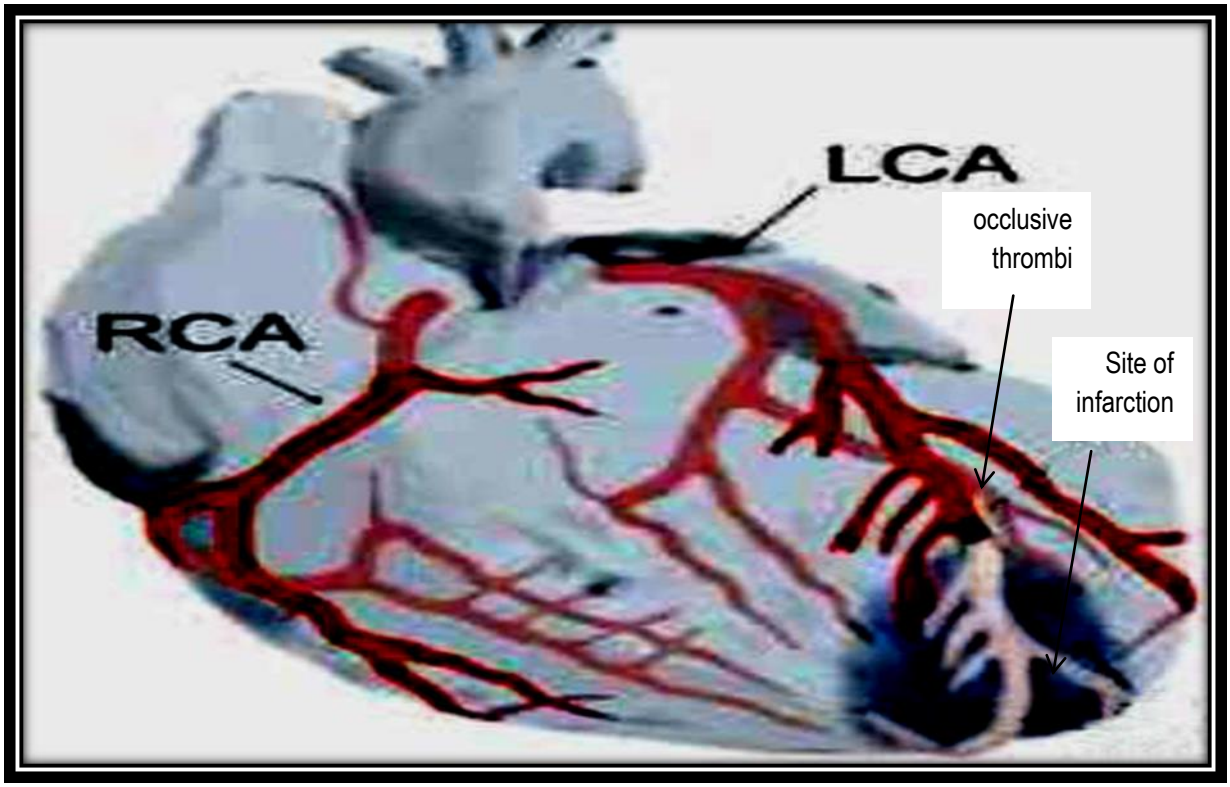


Figure (1-1): Myocardial infarction :show the occlusion of the coronary artery by thrombosis, and necrotic area[24]

Acute myocardial infarction (AMI) is one of the major causes of mortality and morbidity in the world[25]. The most common cause of an AMI is atherosclerotic coronary artery disease(CAD) with erosion or rupture of a plaque causing transient, partial or complete arterial occlusion, the heart cannot continue to function without adequate blood flow, and if it is severely compromised, death is inevitable, several risk factors for coronary heart disease have been well documented, including hypertension, hyperlipidemia, diabetes, a positive family story, smoking, obesity and inactivity[26].

1-2: Unstable angina (UA):

Unstable angina/ NSTEMI is not a specific disease but a clinical syndrome, the classification of unstable angina/ NSTEMI has, until now, been based on simple clinical descriptions, such as whether the ischemic pain is on exertion and accelerating, or whether it occurs at rest[27]. From an etiological perspective, unstable angina /NSTEMI may be classified as thrombosis, severe progressive arterial obstruction, coronary vasospasm/vasoconstriction, inflammation and increased myocardial oxygen consumption[28].

Unstable angina/ NSTEMI manifests under resting conditions, the pain is characteristically longer lasting and more intense (though without exceeding 20-30 minutes),and is less responsive to nitrates, in one-half of all cases of angina the ECG tracing is abnormal, while in the rest of cases ST-segment alterations are evidenced[29].

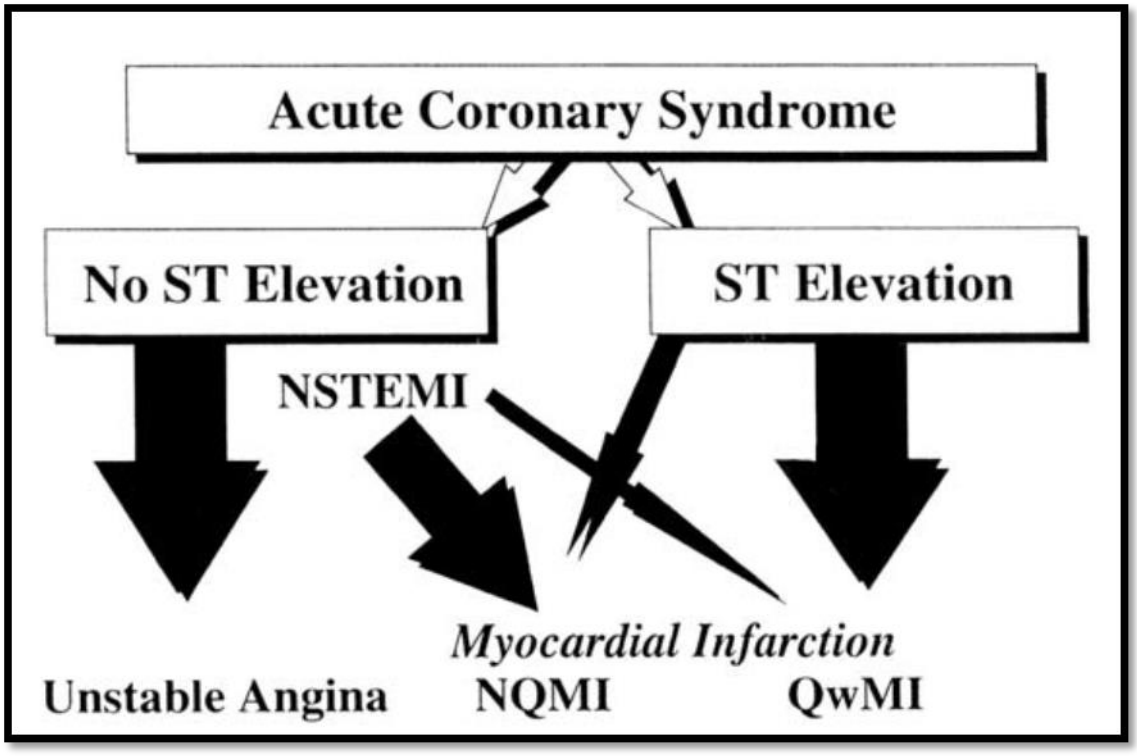


Figure (1-2) Clinical classification of acute coronary syndromes. [30].

1-3: Risk Factors of Acute coronary syndrome:

١-Hyperlipidemia

High cholesterol diet leading to hyperlipidemia is regarded as an important factor in the development of ischemic heart disease, and the focus so far has been mainly on the systemic and coronary vascular effects of cholesterol, although only few studies questioned the effect of cholesterol diet on the heart, several structural and functional alteration have been shown [31,32]. It is well known that the heart of hyperlipidemic/atherosclerotic patients is hardly capable of adapting to physical exercise or other kind of stress, suggesting that the endogenous adaptive mechanisms against myocardial stress are impaired [33].

٢-Hypertension:

High Blood Pressure or hypertension is a risk factor for heart and kidney diseases, as well as strokes, in fact, more than a million heart attacks and a half a million strokes are caused in part by high blood pressure, most people think high blood pressure affects only adults, that's not the case however, for some children, high blood pressure is caused by problems with heart or kidneys and brain [34]. High blood pressure and cholesterol are still major contributors to the national epidemic of cardiovascular disease[35].

٣-Diabetes Mellitus/impaired glucose

Diabetes Mellitus (DM) is a chronic disorder resulting from a number of factors in which an absolute or relative deficiency of insulin or its function occurs, it is projected that by the year 2025, India alone would have 57 million diabetics mainly of type2 diabetes constituting 90% of the diabetic population [36,37].

The most common and life threatening disorder that besets type 2 diabetic subjects is coronary heart disease (CHD), irrespective of the ethnic back ground the risk for CHD among diabetic subjects is greater by 2 to 4- fold compared to non-diabetic subjects[38]. Hyperglycemia, a hallmark of diabetic condition depletes natural antioxidants and facilitates the production of reactive oxygen species (ROS) which has the ability to react with all biological molecules like lipids, proteins, carbohydrates, DNA,…. etc and exert cytotoxic effects on cellular components [39].

٤-Smoking

Smoking harms nearly every organ of the body, it causes many diseases and reduces the health of smokers, in general, Smoking causes coronary heart disease, the leading cause of death in the United States[40]. Cigarette smoking causes reduced circulation by narrowing the blood vessels (arteries) and puts smokers at risk of developing peripheral vascular disease (i.e., obstruction of the large arteries in the arms and legs that can cause a range of problems from pain to tissue loss or gangrene) [40,41].

Compared with nonsmokers, smoking is estimated to increase the risk of coronary heart disease by 2 to 4 times [40,42].

People who smoke are up to four times more likely to die from coronary heart disease than non-smokers[43]. The number of years of smoking and the number of cigarettes smoked increases this risk[44].

Smoking Increases heart rate and decreases the oxygen carried in the blood – with each inhalation of cigarette smoke, oxygen is replaced by carbon monoxide and other gases[44,45].

The side stream smoke is divided into the solid and gas phases, containing higher concentrations of toxic and carcinogenic compounds

and other volatile and semi volatile compounds[46]. Free radicals and oxidants in the gas phase exist in a steady state in which they are continuously formed and destroyed and their concentration increases as the smoke ages[47]. Smoking also makes blood vessels and blood cells sticky, allowing cholesterol and other dangerous fatty material to build up inside them. This is called atherosclerosis. This in turn can lead to raised blood pressure and clot formation[48].

The risk of coronary heart disease is not reduced by smoking low-tar or low-nicotine cigarettes rather than ordinary smoking [43].

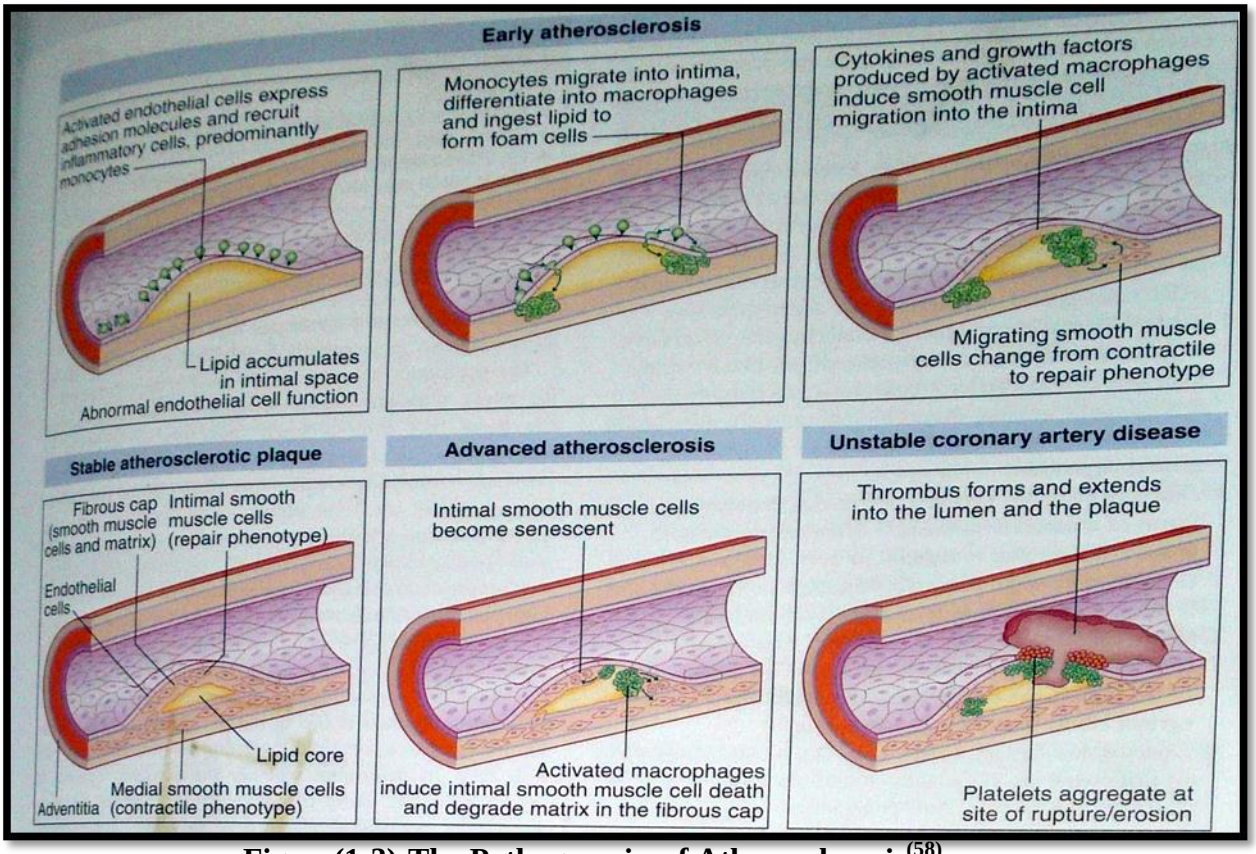
5 - Premature Family History

A positive family history of early heart disease puts subject at increased risk for heart disease, even if subjects don't have other risk factors such as high cholesterol, high blood pressure, diabetes, and obesity[49]**.** Women with a family history of early heart disease have up to a 3-fold higher risk of heart attack and a 5-fold higher risk of dying from heart disease than women with no family history of heart disease.[50,51].

Among people who had a heart attack at age 60 or younger, more women than men have a parent, brother, or sister with heart disease[52,53]**.** If a subject has a sister or brother with heart disease, he is also at higher risk than if he has only a parent with heart disease. This is because in addition to genes, siblings usually grow up with the same life style risk factors (such as bad diet and lack of exercise) [54-56].

1-4:Atherosclerosis:

The development of atherosclerosis follows the endothelial dysfunction, with increased permeability to and accumulation of oxidized lipoproteins, which are taken up by macrophages at focal sites within the endothelium to produce lipid-laden foam cells[57]. Atherosclerosis may manifest as coronary heart disease(e.g. angina ,myocardial infarction, sudden death), cerebrovascular disease(e.g. stroke and transient ischemic attack), occult atherosclerotic vascular disease, intermittent claudication, and is an important cause of subsequent morbidity and mortality in these patients. Atherosclerosis is a progressive inflammatory disorder of the arterial wall that is characterized by focal lipid-rich deposits of atheroma that remain clinically silent until they become large enough to results in thrombotic occlusion or embolization of the affected vassel[58].



Figure(1-3):The Pathogenesis of Atherosclerosis[58].

1-5:CoronaryArtery Diseases(CAD):

CAD is one of the commonest causes of mortality and morbidity all over the world[59].While the symptoms and signs of coronary artery disease are noted in the advanced state of disease, most individuals with coronary artery disease show no evidence of disease for decades as the disease progresses before the first onset of symptoms, often a "sudden" heart attack, finally arises, after decades of progression, some of these athermanous plaques may rupture and (along with the activation of the blood clotting system) start limiting blood flow to the heart muscle, the disease is the most common cause of sudden death[60], and is also the most common reason for death of men and women over 20 years of age[61]. According to present trends in the United States, half of

healthy 40-year-old males will develop CAD in the future, and one in three healthy 40-year-old women[62].

The a etiology of CAD is multifactorial, and a number of risk factors are known to predispose to the condition, some of these-such as age, gender, race and family history cannot be changed, whereas other major risk factors, such as serum cholesterol, smoking habits, diabetes and hypertension, can be modified[57].

1-6:C Reactive protein (CRP):

CRP is not the only inflammatory biomarker that has been shown to predict myocardial infarction and stroke, more sophisticated measures of cytokine activity, cellular adhesion, and immunologic function have all been shown to be elevated among those at increased vascular risk[63]. These approaches, however, are unlikely to have clinical utility because the assays required for their assessment are either inappropriate for routine clinical use or the protein of interest has too short a half-life for clinical evaluation, measures for fibrinogen, a biomarker involved in both inflammation and thrombosis, remain poorly standardized, and methodological issues limit use of this parameter despite consistent population-based data, other broad measures of systemic inflammation, such as the white blood cell count or the erythrocyte sedimentation rate, have proven unreliable in clinical settings. By contrast, high-sensitivity assays for CRP have been standardized across many commercial platforms. Moreover, CRP is highly stable, allowing measures to be made accurately in both fresh and frozen plasma without requirements for special collection procedures. This is due in part to the stable pentraxin structure of CRP and its long plasma half-life of 18 to 20 hours[64].

1-6-1:High Sensitivity C Reactive Protein(hs CRP):

Laboratory and experimental evidence indicate that atherosclerosis, in addition to being a disease of lipid accumulation, also represents a chronic inflammatory process[65]. Thus, researchers have hypothesized that inflammatory markers such as high-sensitivity C-reactive protein (hsCRP) may provide an adjunctive method for global assessment of cardiovascular risk[66-68]. In support of this hypothesis, several large-scale prospective epidemiological studies have shown that plasma levels of hsCRP are a strong independent predictor of risk of future myocardial infarction, stroke, peripheral arterial disease, and vascular death among individuals without known cardiovascular disease[68-78]. In addition, among patients with acute coronary ischemia[79-82] stable angina pectoris[83]. and a history of myocardial infarction[84].

Inflammation plays a major role in atherothrombosis, and measurement of inflammatory markers such as hsCRP may provide a novel method for detecting individuals at high risk of plaque rupture. Several large-scale prospective studies demonstrate that hsCRP is a strong independent predictor of future myocardial infarction and stroke among apparently healthy men and women, several data describing hsCRP within athermatous plaque[85].

hsCRP levels increase with acute infection and trauma[86]. Thus, testing should be avoided within a 2 to 3 weeks in patients who have had an upper respiratory infection or other acute illness. Individuals with clinically apparent inflammatory conditions such as rheumatoid arthritis or lupus are likely to have high level hsCRP well into the clinical range; hsCRP evaluation for the purpose of vascular risk prediction may be of limited value in those patients. However, for most individuals, hsCRP

levels appear to be stable over long periods of time[87]. , effects of low levels of exercise on coronary risk have recently been demonstrated, which is an intriguing issue given that exercise also reduces several inflammatory markers[88]. Diabetic patients have increased levels of hsCRP[89]. which suggests a role for systemic inflammation in diabetogenesis and the insulin resistance syndrome[90,91]. Smokers have elevated levels of hsCRP[92].

1-7: Myocardial Enzymes:

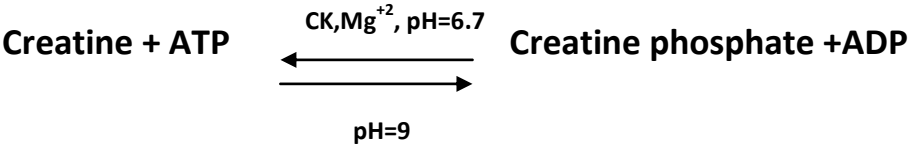
The criteria for the diagnosis of MI were based on the following three features: typical or compatible clinical history, sequential electrocardiographic(ECG) changes; and a rise in cardiac enzymes to at least twice the upper limit of normal for the hospital laboratory. These are the conventional criteria recommended by the *World Health Organization(WHO)* [93]. However, a consistent body of evidence has accumulated to show that these criteria ignore a significant group of patients proven to be at high risk of subsequent cardiac events, in addition, minor rises of creatine kinase (CK)-MB have been demonstrated to be of prognostic significance, and the measurement of cardiac troponins has been shown to be clinically superior to conventional `cardiac enzymes'[94].

1-7-1:Creatine phospho Kinase(CPK-MB isoenzyme):

Creatine kinase (EC.2.7.3.2; Adenosine Triphosphate: Creatine N-phospho-transferase; CK) is a cytosolic enzyme involved in the transfer of energy in muscle metabolism [95]. CK-MB isoenzyme activity assays have been increasingly replaced by CK-MB isoenzyme mass assays that measure the protein concentration of CK-MB isoenzyme rather than its catalytic activity [96,97].

The determination of CK-MB isoenzyme mass has proven to be more specific for myocardial necrosis than the long-standing CK-MB isoenzyme activity and CKMB isoenzyme inhibition assays [98-101]. CK-MB isoenzyme, released after AMI, is detectable in blood as early as 3-4 hours after the onset of symptoms, and remains elevated for approximately 65 hours post infarct [102,103]. CK-MB isoenzyme mass levels are reportedly 50% diagnostic for AMI after 3 hours and > 90% diagnostic at 6 hours [104].

This enzyme catalyzes the reversible phosphorylation of creatine by adenosine triphosphate (ATP), as shown below:



Creatine kinase is highly concentrated in brain, myocardium, and skeletal muscle. It consists of two monomers, each with a molecular weight of 43KDa.The two subunits are termed M (for muscle) and B (for brain) [105].

1-7-2:Lactate Dehydrogenase (LDH):

Lactate dehydrogenase (LDH, EC 1.1.1.27) is a hydrogen transfer enzyme that catalyses the oxidation of L-lactate to pyruvate with nicotine amide-adenine dinucleotide NAD^+ as hydrogen acceptor, the final step in

the metabolic chain of anaerobic glycolysis, the reaction is reversible and the reaction equilibrium strongly favors the reverse reaction, namely the reduction of pyruvate (P) to lactate (L) [106-108].

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

Functional lactate dehydrogenase are homo or hetero tetramers composed of M and H protein subunits encoded by the LDHA and LDHB genes, respectively:

- **LDH-1 (4H) - in the heart and red blood cells**
- **LDH-2 (3H1M) - in the reticuloendothelial system**
- **LDH-3 (2H2M) - in the lungs**
- **LDH-4 (1H3M) - in the kidneys, placenta, and pancreas**
- **LDH-5 (4M) - in the liver and striated muscle[109].**

LD_3 is the main coenzyme elevated due to malignancy of many tissues, also elevation of LD_5 occurs after damage to the liver or skeletal muscles and a rise in LD_1 is most significant in the diagnosis of myocardial infarction [110].

1-8; Free Radicals (FR_S) and Reactive Oxygen Species (ROS):

A free radical is an atom or group of atoms that contains at least one unpaired electron in the outermost shell, and that is capable of independent existence [111]. It is highly reactive and initiates a chain of reaction by extracting an electron from other compounds to complete their own orbital [112].

Free radicals and reactive oxygen species are too reactive to be tolerated in living tissues and their removal and control may have had a

dominating evolutionary pressure with the first appearance of O_2 in the atmosphere, a hierarchy of mechanisms has been evolved to deal with these reactive intermediates [113-115].

Free radicals and reactive oxygen species (ROS) are known to be generated in ischemia and oxidative stress [116]. An antioxidant defense mechanism exists to control an excess of ROS[117]. Primary enzymatic defenses include manganese, copper-zinc superoxide dismutase, catalase, and glutathione peroxidases[118].

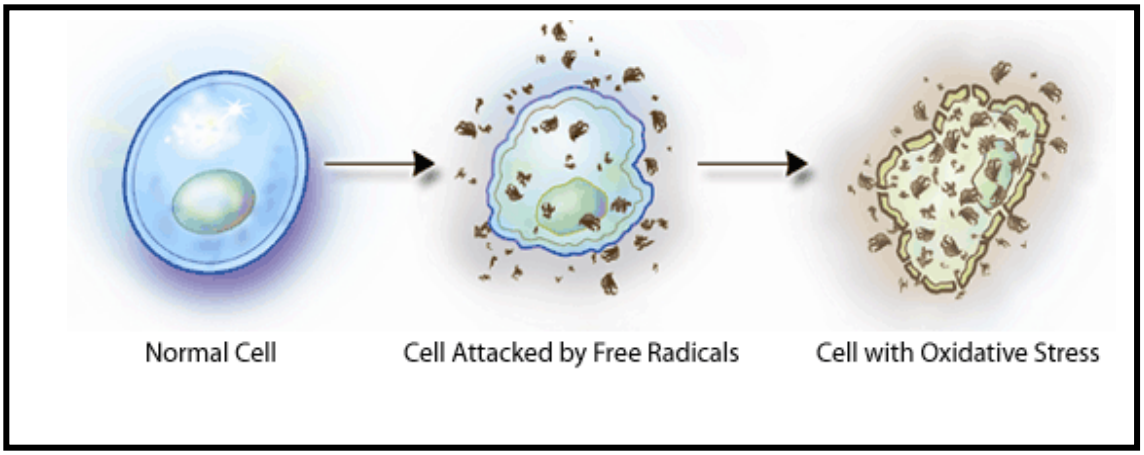


Figure (1-4) Free radicals and oxidative stress[119].

They are oxygen containing compounds that are highly reactive free radicals, or compounds readily converted to these oxygen free radicals in cells [112]. Important ROS in mammalian cells include superoxide anion ($O_2^{-\bullet}$), hydroxyl radicals ($OH^{\bullet}$), and hydrogen peroxide. There is investigation has suggest that the reactive nitrogen species peroxy nitrite ($ONOO^{-}$) and also play an important role in cardiovascular dysfunction [120].

In general, the production of these species may occur enzymatically, or non-enzymatically, as accidental by products or major products of reactions [121]. The superoxide anion which plays a role, as a precursor of other reactive species is produced from one-electron

reduction of oxygen, this process is mediated by enzymes such as NADPH oxidase and xanthine oxidase ,or non-enzymatic by redox-reactive compounds such as the semi-ubiquinone compound of the mitochondrial electron transport chain, as illustrated in Figure (1-5) [122].

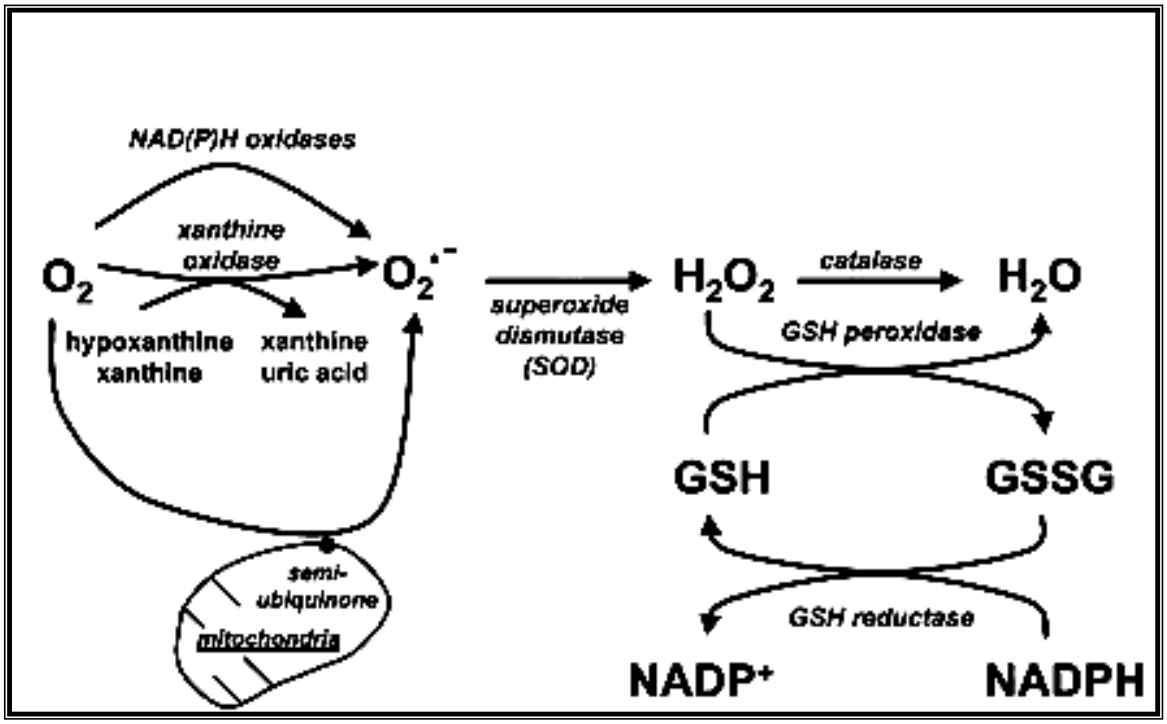


Figure (1-5): Pathways of ROS production and clearance [122].

1-9:Lipid Peroxidation (LPO):

Free radicals may attack many components of a cell such as the polyunsaturated fatty acid (PUFA), deoxyribonucleic acid (DNA) and proteins, the damage of the lipid membrane is called lipid peroxidation, lipid peroxidation is a process related to free radicals whereby in this process, free radicals got hold of electrons from the lipid such as the cellular membrane, this process often affects the polyunsaturated fatty

acid, because they contain multiple double bands in between which lie the methylene-CH_2 groups that contain the reactive oxygen, in the cellular membrane, the oxidative stress is the by -product of the free radicals oxygen [123].

Malonaldehyde (MDA), thiobarbituric acid reactive substance (TBARS), lipid hydroperoxides (LH), and 4-hydroxyalkenals (4-HNE) are the example of lipid peroxidation by-products which have been used as biomarker of lipid peroxidation level, many investigations have shown that MDA, TBARS, LH and 4-HNE are directly linked to increase the rates of lipid peroxidation[124].

$$H_2O_2 + Fe^{2+} \rightarrow \cdot OH + OH^- + Fe^{3+}$$

Oxidative stress is characterized by an increased concentration of oxygen-derived products that provoke critical, even irreversible, cell injury, oxygen reduction leads to the synthesis of reactive intermediate compounds such as the superoxide anion, hydroxyl radical, hydrogen peroxide and peroxidative derivatives of polyunsaturated fatty acids (PUFA) such as conjugated dines, lipid hydroperoxides and malonyldialdehyde (MDA)[125].Oxidation of circulating low density lipoprotein (LDL) has been linked to the initiation and pathogenesis of atherosclerosis and ultimately to the pathogenesis of cardiovascular disease[126].

Oxidative stress alters the plasma lipoprotein profile (particularly LDL), the coagulate parameter (with an increased thrombotic risk), and the cell membranes (which undergo peroxidation)[125].

Oxidative stress can also impair the ability of the endothelium, the inner layer of cells that line the blood vessels, to expand and dilate in response to blood flow, based on this scenario, an accumulation of

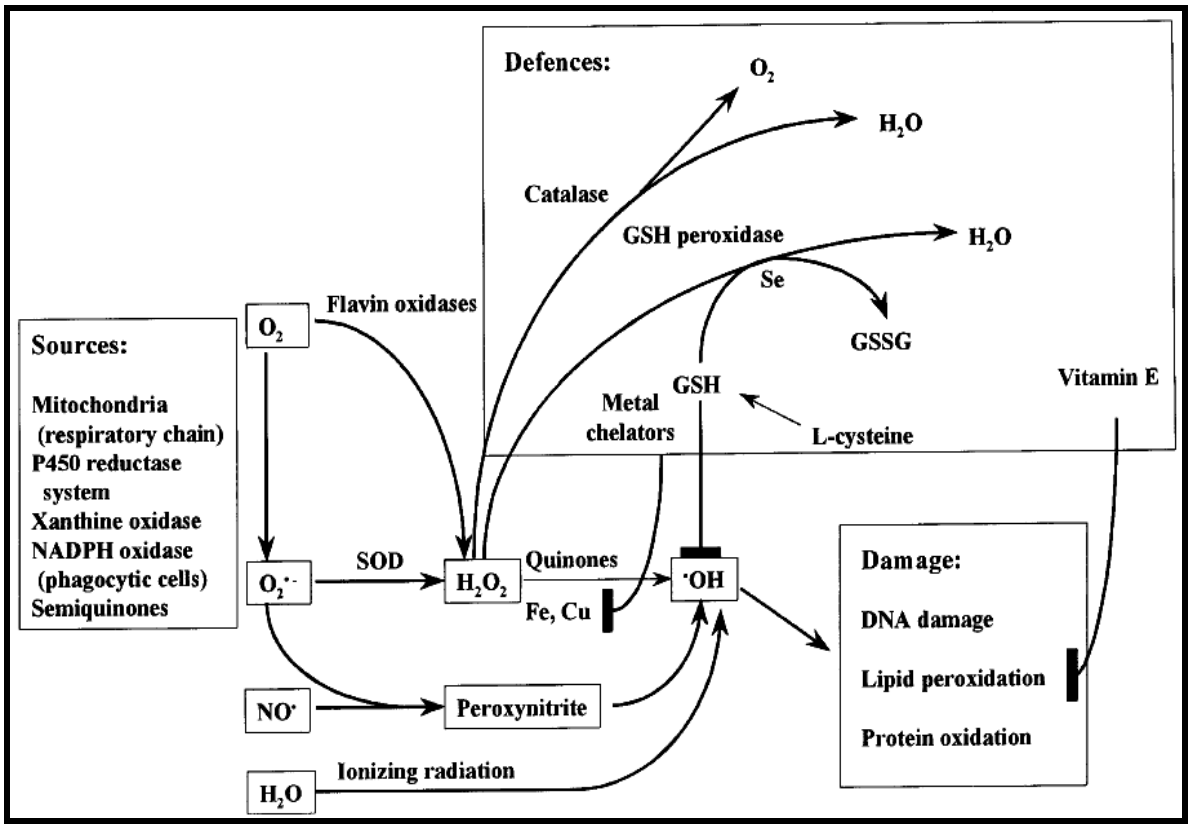
reactive oxygen species has been linked to 'cardiac contractile dysfunction, potentially leading to arrhythmia and heart attack[127].

Table(1-1) Biologically significant free radical [128-130].

Oxidant	Description
$\bullet O_2^-$, superoxide anion	**One-electron reduction state of O_2, formed in many autoxidation reactions and by the electron transport chain. Rather unreactive but can release Fe^{2+} from iron-sulfur proteins and ferritin. Undergoes dismutation to form H_2O_2 spontaneously or by enzymatic catalysis and is a precursor for metal-catalyzed •OH formation.**
H_2O_2, hydrogen peroxide	**Two-electron reduction state, formed by dismutation of $\bullet O_2^-$ or by direct reduction of O_2. Lipid soluble and thus able to diffuse across membranes.**
•OH, hydroxyl radical	**Three-electron reduction state, formed by Fenton reaction and decomposition of peroxynitrite. Extremely reactive, will attack most cellular components**
ROOH, organic hydroperoxide	**Formed by radical reactions with cellular components such as lipids and nucleobases.**
RO•, alkoxy and ROO•, peroxy radicals	**Oxygen centered organic radicals. Lipid forms participate in lipid peroxidation reactions. Produced in the presence of oxygen by radical addition to double bonds or hydrogen abstraction.**
HOCl, hypochlorous acid	**Formed from H_2O_2 by myeloperoxidase. Lipid soluble and highly reactive. Will readily oxidize protein constituents, including thiol groups, amino groups and methionine.**
ONOO-, peroxynitrite	**Formed in a rapid reaction between $\bullet O_2^-$ and NO•. Lipid soluble and similar in reactivity to hypochlorous acid. Protonation forms peroxynitrous acid, which can undergo hemolytic cleavage to form hydroxyl radical and nitrogen dioxide.**

1-10:Antioxidants:

Antioxidant molecule can prevent the oxidation of other molecule Oxidation is a chemical reaction that transfers an electron from a molecule to an oxidizing agent, oxidation reaction can produce free radicals which cause cellular damage, antioxidants terminate this chain reaction by removing the free radical species and inhibit other oxidation reactions by oxidizing themselves[124]. There are two major antioxidant defense systems to protect the body against the reactive oxygen species and cellular damage, which are the enzymatic and non- enzymatic antioxidants, the non-enzymatic system includes the glutathione, uric acid, vitamin C, and vitamin E, antioxidant enzymes are also produced by the body which include catalase, superoxide dismutase, and glutathione peroxidase [131]. These two types of antioxidant system are working together to ameliorate any harmful effects of oxidant in the cell, both of the enzymatic and non-enzymatic antioxidants detoxify ROS in the intracellular and extracellular environments[112].There are a lot of interests in the effects of antioxidant supplement both in terms of promoting performance and also preventing tissues damage, which occur during exercise, particularly for those who undertake irregular and strenuous activity[132]. the major ROS pathways and antioxidants are illustrated in figure (1-6).



Figure(1-6). Major reactive oxygen species pathways and antioxidant defenses.. Abbreviations: GSH, reduced glutathione; GSSG, oxidized glutathione; SOD, superoxide dismutase[133].

1-10-1:Ceruloplasmin (Cp):

It is an important protein that circulates in plasma as a major copper ion transporter, (90-95%) of human plasma copper is associated with the ceruloplasmin as a no dialyzable fraction and the remaining 5-10% of plasma copper is fairly loosely attached to albumin and histidine, but only a trace of copper is present as free Cu^{++}[134,135]. Normally, ceruloplasmin is synthesized in the liver, and secreted into plasma [136].

Ceruloplasmin, the multifunctional copper containing enzyme, possesses a significant oxidase activity directed toward ferrous ions [137,138].

The oxidase activity increases during inflammation, infection, and injury which suggests that serum Cp possibly acts as an antioxidant and as an acute phase protein [139]. It is implied that during exposure to oxidative stress, substantial inactivation of Cp may occur and free copper ion may be released [140,141]. Therefore, the damaged Cp may cause the augmentation of free radical-mediated damage to other macromolecules upon exposure to oxidative stress, thus, Cp is a very important component of the cellular defense mechanism against toxicity [142].

1-10-2:Transferrin(Tf):

Serum iron, total iron-binding capacity (TIBC), and calculated transferrin saturation (TS) tests are used to screen for and monitor conditions of iron deficiency and iron overload, although the usefulness of these tests for diagnosing iron deficiency can be debated[143].

Transferrin is a major plasma protein of biological interest because of its evolutionary history and because of its multi regulatory control[144].Transferrin synthesis and storage are regulated by iron levels, estrogens, and nutritional status [145]. Transferrin transports ferric iron into cells by receptor-mediated endocytosis, a unique process by which transferrin and its receptor are reutilized repeatedly in iron delivery, in humans, serum transferrin concentration decreases in iron overload and increases in chronic iron deficiency[146,147].

Transferrins are involved in iron regulation and transport in vertebrates and some invertebrates such as worms or insects [148].

Metal binding proteins, targeted to bind iron and copper ions, ensure that these Fenton metals are cryptic in all hereditary disorders with the higher potential for oxidative damage due to chronic redox imbalance in red cell that often results in clinical manifestation of mild to serve hemolysis in patients with these disorders [149].

1-11:Lipids and Lipoproteins:

1-11-1:Lipids:

Lipids are a class of nonpolar molecules. They are found in the cell membranes, in the endoplasmic reticulum, and in specialized fat storage cells [150]. Plasma lipids consist of triacylglycerol (triglycerides) 16%, phospholipids 30%, cholesterol 14%, cholesterol esters 36% and a much smaller fraction of un esterified long-chain fatty acids or free fatty acids 4% [151]. The clinically important lipids are illustrated in Table (1-2)

Table (1-2):The classification of clinically important lipids[134].

Sterol derivatives	**Glycerol esters**
Cholesterol and cholesterol esters	**Triglycerides(Triacylglycerol)**
Steroid hormones	**Phosphoglycerides**
Bile acids	**Sphingosine derivatives**
Vitamin D	**Sphingomyelin**
Fatty acids	**Glycosphingolipids**
Short chain (2 to 4 carbon atoms)	**Terpenes (isoprene polymers)**
Medium chain (6 to 10 carbon atoms)	**Vitamin A**
Long chain (12 to 20 carbon atoms)	**Vitamin E**
Prostaglandins.	**Vitamin K**

1-11-1-1:Cholesterol

It is a member of a large group of substances called steroids. It is the precursor for the synthesis of many physiologically important steroids, such as bile acids and steroid hormones [152]. Although a portion of the body's cholesterol is derived from dietary intake, most of it is synthesized by the liver and other tissues from simpler molecules, almost 90% of synthesis occurs in the liver and gut; therefore, peripheral cells and other organs depend largely on cholesterol delivery from the circulation [135].

1-11-1-2:Triglyceride

Triglycerides are fatty acid esters of glycerol, each containing three different fatty acids [152]. They are derived from three primary sources: the diet, storage depots in adipocytes and biosynthesis particularly in the liver [150]. Triglyceride are hydrolyzed into the glycerol and fatty acids, and after absorption, triglyceride are synthesized in the epithelial cells [135].

1-11-2:Lipoproteins:

It includes any of the lipid-protein complexes in which lipids are transported in the blood. Lipoprotein particles consist of a spherical hydrophobic core of triglycerides or cholesterol esters surrounded by an amphipathic monolayer of phospholipids, cholesterol, and apolipoproteins, lipoproteins in the blood, a water medium, carry fats around the body[153].

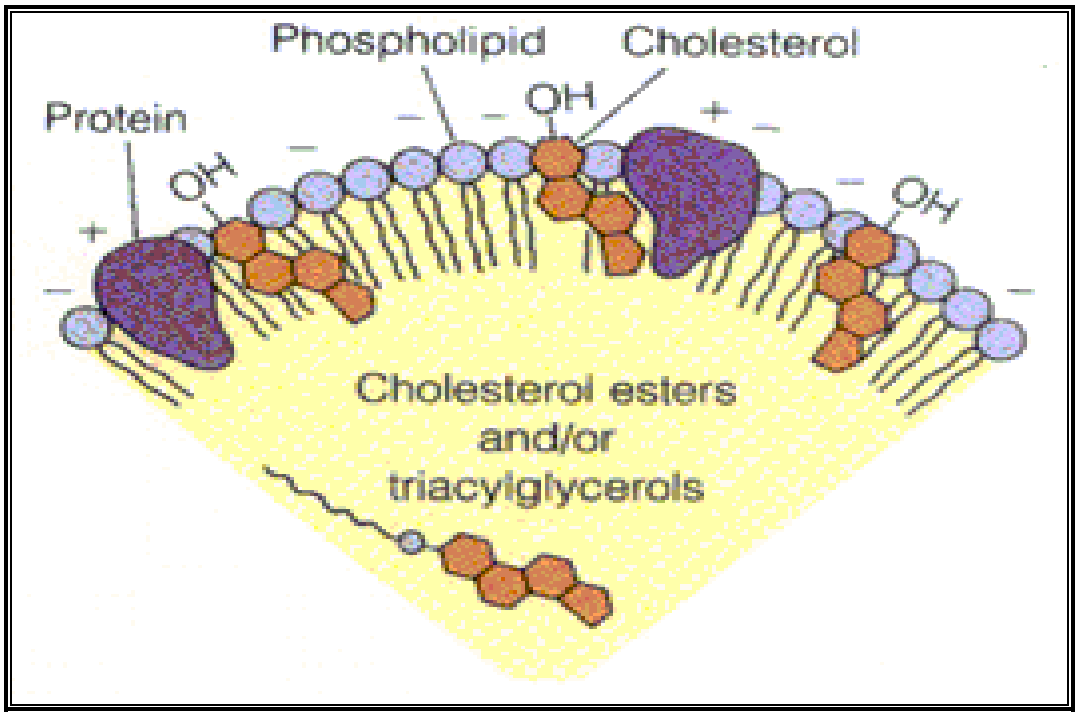


Figure (1-7):The generalized structure of plasma lipoprotein(150).

1-11-2-1: High Density Lipoprotein (HDL):

It is produced in the liver and the intestine and it exchanges proteins, lipids with other lipoproteins, it functions in the return of cholesterol from peripheral tissues to the liver and migrated to α-globulin region.

1-11-2-2:Low Density Lipoprotein (LDL):

It is produced in blood (remnant of IDL after triacylglycerol digestion; end product of VLDL), it represents the primary carriers of cholesterol in the blood for delivery to all peripheral tissues and endocytosis by liver and peripheral tissues and contains high concentration of cholesterol and cholesterol esters then migrate into β-globulin region.

1-11-2-3:Intermediate Density Lipoprotein (IDL):

It is produced in the blood (remnant of VLDL after triacylglycerol digestion) and endocytosed by liver or converted to LDL.

1-11-2-4: Very Low Density Lipoprotein(VLDL):

It is produced in liver mainly from dietary carbohydrate and chief carrier of endogenous hepatic triacylglycerol in blood, then migrate into pre-β-globulin region in electrophoretic field.

1-11-2-5:Chylomicrons(CM) :

It is produced in the intestinal epithelial cells from dietary fat then chief carrier of exogenous triacylglycerol in blood and appear in origin in electrophoretic field, of the five lipoprotein classes, LDL is the richest in cholesterol. When endothelial cells engulf LDL, they oxidized it to a product called oxidized LDL [154,155].

1-12:Atherogenic Index:

Many anthropometric, clinical and biochemical factors can influence the composition and size of lipoprotein subpopulations. It has been demonstrated that the prevalence of small dense LDL particles increases cardiovascular (CV) risk [156-158]. and that the distribution of differently sized particles in HDL influences its anti-atherogenic effects [159-163]. There are two established markers of CV risk, namely FERHDL (cholesterol esterification rate in Apo B-depleted plasma) and AIP-atherogenic index of plasma [Log (TG/HDL-C)] reflect the size of LDL and HDL subpopulations and closely correlate with each other over a wide range of plasma lipid values. AIP is, of course, a transformation of TG/HDL-C that better meets the assumption of normality of the errors in

the statistical model being used to describe the treatment effects than does the untransformed variable[164,165].

Disorders of lipoprotein metabolism are often implicated in pro-atherogenic processes which can ultimately result in cardiovascular disease. Increased levels of low-density lipoprotein cholesterol (LDL-C), reduced levels of high density lipoprotein cholesterol (HDL-C) and increased plasma triglyceride (TG) levels are recognized as independent risk factors for cardiovascular morbidity**,** lipoprotein disorders are frequently encountered in clinical practice, their main effect on health is to increase cardiovascular risk and, in the case of extremes of triglyceride elevations, cause pancreatitis, while knowledge of the genes involved in lipoprotein metabolism has greatly increased this knowledge of metabolism, cellular biology, genetics, epidemiology and nutrition, treatment of lipoprotein disorders should primarily involve a healthy lifestyle; medications are indicated in severe cases and in patients at increased cardiovascular risk [166-168].

1-13: Aim of Study:

This study is designed to provide information on the following aims:-

1-Evaluation of serum (high sensitivity C reactive protein) and (C reactive protein) in Acute coronary syndrome (Acute myocardial Infarction and Unstable angina /NSTEMI).

2-Confirmation serum myocardial enzymes in the mention diseases by measurements (Creatine phospho kinase(CPK-MB isoenzyme), Lactate dehydrogenase (LDH)).

3- Evaluation of serum oxidants and antioxidants status in the mentioned diseases .

4-Investigation of serum lipid profile and athergenic index in the mentioned diseases.

5-Sheding a light on the possible correlation relationships between high sensitivity C reactive protein and each one of the studied parameters.

CHAPTER TWO

METHODS AND MATERIALS

CHAPTER TWO

MATERIALS AND METHODS

2.1. Design of Study:

This study conducted at AL-Hussein Teaching Hospital in Thi-Qar, especially, in the coronary care unit (CCU) , Biochemistry Laboratory, the Hormones and immunes Laboratory and specialist clinics at the period between 6/10/2011 to 1/5/2012. It included (170) subjects, control(70) and patients(100).

Table (2-1): Data of patients and controls groups

Groups	n
Patients	100
Controls	70

The study has been conducted on total number of supposed healthy individuals and patients who were divided into:-

AMI group : 55 patients with Acute Myocardial Infarction (AMI) [40 males and 15females] with age range (42—78).

UA group : 45 patients with Unstable Angina /NSTEMI (UA) [32 males and 13 females] with age range (39—80).

control group : control group, consist of 70 supposed healthy subjects [47 males and 23 females] with no history of systematic illness at age range (35 −80).

The period of time for patients with AMI and UA /NSTEMI who took them blood samples ranged from (1-10) days.

2-2:Collection of Blood Samples:

About(8mL) of blood samples of acute myocardial infarction(AMI), unstable angina/NSTEMI (UA) patients and controls were taken and allowed to clot at room temperature in empty disposable tubes centrifuge to separate it in the centrifuge at 3000 rotor per minute (rpm)for 10min,the serum samples were separated and stored at (-20ºC) for later measurement of biochemical parameters, unless used immediately.

The clinical chart of this study describes experimental parameters in the following table (2-2).

Table(2-2):The clinical chart of the study

Patient profile
Name:-
Age:-
Sex:-
Occupation:-
Address:-
DX: a:AM I b:UA
Risk factors:- a:HTN b:DM c:smoking d:hyperlipidmip e:family history
Drugs:-
Biochemical tests
C reactive protein (CRP), High sensitivity C reactive protein(hsCRP)
Myocardial Enzymes:-CPK, LDH

Lipid peroxidation:- MDA
Antioxidants:- CP , Tf
Lipid Profile:-TCH,TG, HDL ,LDL, VLDL
Atherogenic Index:- AI

2-3:Chemicals:

The chemicals used in this study with their companies which are showed with suppliers are shown in table(2-3).

Table (2-3):Chemicals and their suppliers

Chemicals	Suppliers
Butanol	**BDH, England**
C reactive protein(CRP)	**Plasmatic, England**
Cholesterol Kit	**Spinreact, Spain**
Creatine phospho kinase(CPK)	**Biolabo, France**
Glcail acide	**BDH, England**
HDL Kit	**Biomerieux, France**
High sensitivity C reactive protein(hsCRP)	**Demeditec, Germany**
Iron Kit	**Biolabo, France**
Lactate dehydrogenase(LDH)	**Biomagrab, Tunisia**
Para pheneline di amine(PPD)	**BDH, England**

Sodium acetate	**BDH, England**
Sodium azide	**Reidledehain, Germany**
Thio bar bituric acid(TBA)	**BDH, England**
Total Iron Binding Capacity(TIBC)Kit	**Biolabo, France**
Tri chloro acetic acid(TCA)	**BDH, England**
Triglyceride Kit	**Biolabo, France**

2-4:Instruments:

The instruments are available in clinical biochemistry laboratory in AL-Hussein Teaching Hospital and laboratories of chemistry department in the college of science (University of Thi-Qar) which are shown in table(2-4).

Table(2-4):The instruments and their manufacturers

Instruments	Manufacturers
Centrifuge	**Universal 16A , Germany**
Elisa	**Bio Tek , P.N 7331000 ,USA**
Incubator	**Jard, Japan**
Sensitive balance	**MO.12307078, Japan**
Shacking water bath	**SW23,Germany**
UV/VIS spectrophotometer	**UV-7804C, Japan**
UV/VIS spectrophotometer	**T 60,PG Instruments Ltd, Germany**

2-5:Biochemical Parameters:

Several considerable methods were used to measure the studied parameters.It is notable that all measurements were duplicated for each sample.

2-5-1:Determination of serum C reactive protein(CRP) Concentrations: [169,170]

Principle:

The assay was performed by testing a suspension of latex particles coated with anti-human CRP antibodies against unknown serum. The presence of visible agglutination indicates an increase in levels of CRP to a clinically significant level.

Composition:

Standard Kit contents: may vary depending on format supplied Latex reagent sufficient for **50/100** slide tests using re-usable dropper as supplied. Contains <0.1% Azide.

Positive Control. This serum was human positive CRP serum with a concentration >15mg/L. This reagent was ready for use and will give positive results when tested with the CRP latex test. contains < 0.1% Azide.

Negative control. This control was a negative CRP control serum. This reagent was ready for use and will give a negative result when tested with the CRP latex reagent . contains <0.1% Azide.

10x Concentrate. Glycine Diluent Buffer. Add one part to nine parts distilled water before use. On dilution the diluent has a pH between 8.0 and 8.2. contains <0.1%Azide .

Procedure:

Qualitative method

1- Each component was allowed to reach room temperature.

2- The latex reagent was gently shaken disperse the particles.

3- One drop was added to the latex reagent using the dropper provided (40µL) to each of the required circles of the agglutination slide.

4- The pipette was used stirrer provided, place a drop of undiluted serum into a circle of a test slide.

5- the reagent was spread and serum sample over the entire area of the test circle using a separate stirrer for each sample.

6- The test slide was gently tilted backwards and forwards approximately once every two seconds for two minutes. The results were Interpreted immediately after 2 minutes. Extended incubation may lead to false results. Positive and negative controls should be included at regular intervals. Both were ready for used and did not required further dilution.

7-At the end of the test rinse the test slide with distilled water, dried and stored in a sealed bag. Normal laboratory precautions should be maintained while handling potentially infectious patient samples

2-5-2:Determination of Serum High Sensitivity C Reactive Protein Concentrations: [171,172]

Principle of the CRP Elisa:

Microtiterstrips coated with anti-CRP antibody were incubated with diluted standard sera and patient samples. During this incubation step CRP was bounded specifically to the wells .After removal of the unbound serum proteins by a washing procedure, the antigen – antibody complex in each well was detected with specific peroxidase – conjugated antibodies.

After removing of the unbound conjugate, the strips were incubated with a chromogen solution containing tetramethylbenzidin and hydrogen peroxidase: a blue colour develops in pro protein to the amount of immune complex bound to the wells of the strips. The enzymatic reaction is stopped by the addition of (1N) acidic solution and the absorbance values at 450nm were determined .

A standard curve was obtained by plotting the absorbance values versus the corresponding standard values. The concentration of CRP in patient samples is determined by interpolation from the standard curve.

Reagents:

1-Coated Micro titer strips -12x 8-well strips coated with monoclonal antibodies to human CRP.

2-Standard Sera -5 vial, each containing 1/10 prediluted CRP standard solutions (0.2mL): 0 – 0.4-1-5-10 µ g/mL . Calibrated against the NIBSC 1st International standard , 85/506.Contain 0. 09% NaN_3 and antimicrobial agents as preservatives.

3-Conjugate -1 vial, containing peroxidase conjugated monoclonal anti-human CRP antibodies (12mL).contains antimicrobial agents and an inert red dye .

4-Specimen Dilution Buffer -1 vial, containing 40 mL dilution buffer 5x concentrated. Contains 0.09% NaN_3 and antimicrobial agents and an inert green dye.

5-Washing Solution -1 vial containing 50 mL 20x concentrated phosphate buffered washing solution.

6-Chromogen Solution: 1 vial, containing 15 mL of a solution containing H_2O_2 and tetramethyl benzidin.

7-Stopping Solution -1 vial, containing 12 mL of 1N acidic solution.

Assay Procedure

Reconstitution of the Reagents:

Washing Solution: 50mL of concentrated washing solution (**5**) was dilute to 1000 mL with distilled water. Reconstituted solution can be stored at least 1 month, store at 2-8^{o}C.

At higher temperatures, the concentrated washing solution (**5**) may appear cloudy without affecting its performance. Upon dilution, the solution was clear.

Sample diluent: 40 mL of the concentrated sample diluent was Dilute to 200 mL with distilled water. Reconstituted solution can be stored at least 3 months or as long as solution remains clear. It was Stored at 2-8^{o}C.

Assay Procedure:

1-The 10 x pre diluted standard sera (**2**) were diluted 1:100 as follows: 10µL was pipetted of each calibrator into separate glass

dilution tubes. 990µL was added of diluted specimen dilution buffer(**4**) and mixed carefully.

2-The patient samples were diluted 1:1000 in two consecutive steps: 10µL was pipetted of each patient sample into separate glass dilution tubes and 990µL was added of diluted specimen dilution buffer(**4**). Mixed thoroughly. 450 µL was added to diluted specimen dilution buffer to 50µL of these 100 x pre diluted samples. Mixed thoroughly.

Warning: do not store the diluted samples for more than 8 hours.

3- 100µL was pipetted of the diluted calibrators and samples into each of a pair of adjacent wells(**1**).

4- The covered micro titer strips were incubated for **30** ± 2 min at room temperature.

5- the micro titer strips were washed three times with washing solution. This can either be performed with a suitable microtiterplate washer or by briskly shaking out the contents of the strips and immersing them in washing solution. During the third step, the washing solution was left in the strips for 2-3 min. change washing solution for each cycle. Finally the micro titer strips were empty and removed excess fluid by blotting the inverted strips on adsorbent pa per.

6- 100µL was added of Conjugate Solution (**3**) and the covered micro titer strips were incubated for 30±2 min at room temperature.

7- the washing procedure was repeated as described in **5**.

8- 100µL was added to Chromogen solution (**6**) to each well.

9-Incubated for 10±2 min at room temperature. Avoided light exposure during this step.

10- 50µL was added to Stopping Solution (**7**) to each well.

11- The absorbance was determined for each well at **450nm** within 30 min following the addition of acid.

Interpretation of the results:

The following criteria are commonly found in the literature of the relation between the hsCRP values and the risk for developing CVD.

hs CRP values<1.0mg/L=Low risk for CVD.

hsCRP values 1.0-2.9mg/L=Intermediate risk for CVD.

hs CRP values >3.0mg/L=High risk for CVD.

2-5-3:Determination of serum Creatine phospho Kinase(CPK) Activity: [173-175]

Principle:

Enzymatic method described by Oliver modified by Rosalki and later by Szasz.

Phospho creatine + ADP $\xleftrightarrow{CPK}$ Creatine + ATP

ATP + D – Glucose $\xleftrightarrow{HK}$ ADP + D – Glucose – 6 – phosphate

D – Glucose – 6 – phosphate + $NADP^+$ $\xrightarrow{G-6-P\ DH}$ D – Gluconate –6– phosphate + NADPH + H^+

The increase in absorbance, proportional to CK activity in the specimen, was measured at 340nm.

Reagents composition:

Working reagent composition according to IFCC recommendation.

AMP(Adenosine 5 monophosphate) ……..5mmol/l.

NADP(Nicotine amideadenine dinucleotide phosphate)…..2mmol/l.

AP5A(Diadenosine 5 penta phosphate)……10mmol/l.

EDTA(Ethylen diamine tetra acetic acid)……2mmol/l.

Mg+2…….10mmol/l.

ADP(Adenine dinucleotide phosphate)……..2mmol/l.

D-Glucose……..20mmol/l.

N-Acetyl-L-cysteine……20mmol/l.

HK(Hexokinase)……>3000UI/L.

G-6-PDH(Glucose-6-phosphate dehydrogenase)….>2500UI/L.

Imidazole Acetate pH 6.7……100mmol/l.

Creatine phosphate……..30mmol/l.

Contains also surfactants and stabilizers[176].

Manual procedure:

stand reagents and specimens were left at room temperature.

Pipette into 1cm path length thermo stated cuvette
Working reagent…….1mL
Bring to(37ºC), then add:
Specimen…….5oµL
Mixed. Started a timer and recorded initial absorbance at 340nm after 2 min. the absorbance was recorded again every minute during 3 minutes.
Absorbance was calculated change per minute (Δabc/min).

Calculation:

T he result was Calculated as follows:

With theroretical factor:

IU/L=(Δ abc/min) x 3333

2-5-4:Determination of serum Lactate Dehydrogenase(LDH) Activity: (177-179).

Principle:

Kinetic determination of the Lactate dehydrogenase activity optimized test according to the recommendation of SFBC (societe francaise de biologie Clinique).

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

The activity of LDH was shown by the variation of OD at 340nm which was proportional to the quantity of NADH oxidized.

Reagents:

Reagent 1		
	This buffer pH 7.2 at 30ºC	**80mmol/l**
	Buffer reagent pyruvate	**1.6mmol/l**
	NaCl	**200mmol/l**
Reagent 2 Coenzyme	**NADH**	**0.2mmol/l**

Procedure:

Wavelength: ……340nm.

Temperature: ……30-37ºC .

Cuvette: ……1 cm light path.

Zero ajdustement: air or distilled water.

Working Reagent	**1mL**	**3mL**
Equilibrate at choosed temperature		
Sample	**20µL**	**60µL**
Mixed and wait 1 min. **Measured the extinction decrease per min .for 1-3.**		

Calculation:

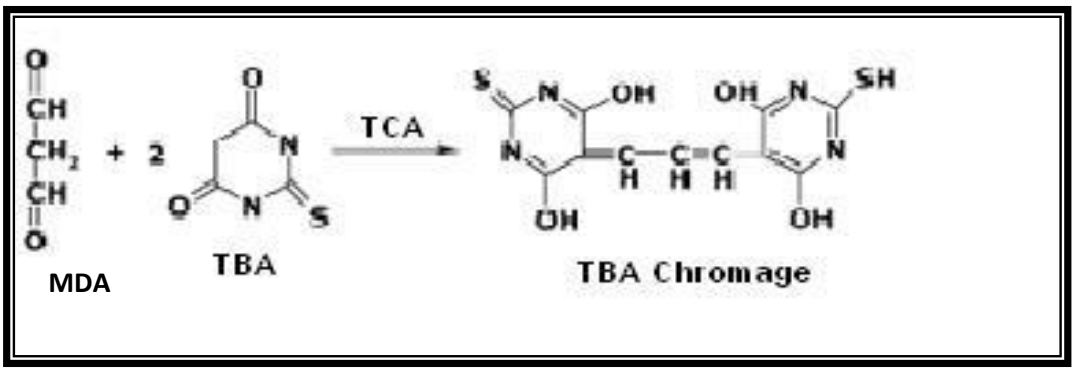
340nm **Δ OD /min x 8095 = U/I**

2-5-5: Determination of Serum Malondialdehyde:

Principle:

Lipid peroxidation products were one of the key indicators of oxidative stress[180]. Lipid peroxidation was determined by using the thiobarbituric acid method[181]. In this method, MDA was formed by degeneration of polyunsaturated fatty acids as the product of LPO that reacts with thiobarbituric acid (TBA), in coexisting trichloro acetic acid (TCA), to give a red chromophore absorbing at 532nm.

MDA concentrations were calculated using the extinction coefficient of MDA ($\mathcal{E}_{MDA}$)equal to 1.56 x10^3 mol^{-1}. cm^{-1}.



Procedure:-

MDA level of the plasma was measured according to a modified method of Fong *et al*[181].

	Sample	Blank
Plasma	0.5 mL	----------
TCA	2.5 mL	----------
TBA	1mL	----------

Mixed, shaked well after each addition and waited for 30 min in a boiling water bath followed by rapid cooling

Butanol	4 mL	4 mL

Centrifuged at 3000 rpm for at least 10 min and read the absorbance of clear supernatant against blank at (535)nm.

The concentration of plasma MDA was computed according to the following equation :

$$\textbf{Plasma MDA (nmol /mL)} = \frac{A_{sample} - A_{blank}}{\varepsilon_{MDA}} = \frac{\Delta A}{1.56} \times 10^3$$

2-5-6:-Determination of Serum Ceruloplasmin Concentrations:

Principle:-

Ceruloplasmin concentration in serum was measured by Menden *et al*[182]. It was based on the ceruloplasmin-catalyzed oxidation of colorless para-phenylene diamine (PPD) to blue-violet oxidize form. The reaction was followed photometrically and the blank value was determined after inhibition of the enzyme with sodium azide at (0^{o}C).

$$\textit{Reduced PPD} + 2H^{+} + \tfrac{1}{2} O_2 \leftrightarrow \textit{Oxidized PPD} + H_2O$$

(colorless) *(blue)*

A mixture of serum, substrate and acetate buffer at pH = 6.0 was incubated at 37°C for 15 min. The reaction was stopped by the addition of sodium azide, and the absorbance of the purple color formed (Oxidized PPD) in the diluted test mixture was read at 525 nm against blank solution. The corrected absorbance was directly related to the concentration of Cp[183].

Reagents :-

● Substrate solution :-

50 mg of PPD was dissolved in 5 mL(4 mL of distilled water (D.W) and 1 mL of glacial acetic acid). In another container 8.15gm sodium acetate tri hydrate was dissolved in 30 mL of D.W then added to the first solution, mixed and completed the volume to 50 mL with D.W.

This solution was stable for three hours and was kept refrigerated in a dark bottle[182].

● ***Inactivating solution :-***

100 mg of sodium azide was dissolved in (500 mL) of D.W then was kept cold in refrigerator prior to use, this solution must be prepared every week.

Procedure :-

- 1 mL of substrate was pipetted into test tubes, incubated at 37°C for 1 min.
- 0.1 mL of plasma was added, incubated at 37°C for 15 min, then tubes were removed and placed in iced-bath for 30 min.
- 5 mL of cold inactivating solution was added, mixed and the temperature was brought to 25°C in water bath.
- Blank was prepared by combining the substrate, inactivating solution, and 0.1 mL of D.W, then the mixture was incubated as above.
- The absorbance of test and blank was read in spectrophotometer at 525nm.
- The concentration of Cp was calculated using the extinction
- coefficient of Cp ($\mathcal{E}_{Cp}$) equal to (0.68).

$$\textbf{Serum Cp (gm/L)} = \frac{A_{test} - A_{blank}}{\mathcal{E}_{Cp}} \times 10 = \frac{\Delta A \times 10}{0.68}$$

2-5-7:Determination of serum Transferrin concentrations:

2-5-7-1:Total Iron Binding Capacity(T.I.B.C.): [184,185].

Principle:

T.I.B.C. was determined by addition of sufficient Fe^{+3} to saturate iron binding sites on apo transferrin . the excess Fe^{+3} was removed by adsorption with basic magnesium carbonate powder.

After centrifugation, bound iron remaining in supernatant was measured with direct method {REF} 92108.

Reagents:

{Vial R1} Iron solution

Hydrochloric acid ……5mmol/L.

Ferric chloride≥ 502 µg/dL (≥ 90µmol/L.

{Packet R2} Preciptant

Magnesium carbonate ……150 mg/capsule.

Manual Procedure:

stand reagents and specimens were let at room temperature.

The following components were pipetted in centrifuge tube	
Specimen(*)	1mL
Iron solution (vialR1)	2mL
Mix , wait 10 minutes then add:	
Precipitant(capsule R2)	150mg (The contents of a capsule)

Mixed well by inversion. Allowed to stand at room temperature for 30 min. shaking on a rotary or swinging agitator. Centrifuge 10 min at 3000rpm.
Iron in supernatant was measured, with reagent {REF}92108.

Calculation:

1- The result was calculated as in the reagent technical data sheet used for iron determination.

2- T.I.B.C. was calculated as follows:

T.I.B.C.=Iron concentration measured in supernatant x3

(dilution 1ml specimen /2ml iron solution).

Units conversion factor :µmol/L=µg/dLx0.1792.

2-5-7-2: Iron: [186]

Principle:

After dissociation of iron-transferrin bound in acid medium, ascorbic acid reduces Fe^{+3} iron into Fe^{+2} iron. Fe^{+2} iron then form a coloured complex with 3-(2-pyridyl) -5, -6 difuryl-1, -2,-4triazine-disulfonate (Ferene). The absorbance thus measured at 600nm (580-620) was directly proportional to the amount of iron in the specimen. Thiourea was added in the reagent to prevent the copper interference.

Reagents Composition:

{vial R1} Reductant

Citric acid ……..150mmol/L.

Ascorbic acid …….30mmol/L.

Thiourea ……27mmol/L.

{Vial R2} Chromogen

Ferene ……600µmol/L.

{Vial R3} Standard

Iron 200µg/dL(35.8µmol/L)

Manual procedure:

Stand reagents and specimens were let at room temperature. Prepare 2 sets of tubes according to following boards:

Blank- Tubes	**Blank**	**Standard**	**Assay**
Reagent R1	**1mL**	**1mL**	**1mL**
Specimen			**200µL**
Standard		**200µL**	
Distilled water	**200µL**		

Mixed gently . Let stand for at least 3 mins at room temperature.

Recorded A_1 absorbances at 600 nm(580-620) against blank.

Assay- Tubes	Blank	Standard	Assay
Working Reagent	**1mL**	**1mL**	**1mL**
Specimen			**200µL**
Standard		**200µL**	
Distilled water	**200µL**		

Mixed

gently. Let stand for 5 mins at room temperature

Recorded A_2 absorbances at 600nm(580-620) against blank.

Colour was stable for 1 hour.

Calculation:

Calculate the result as follows:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standarad)}} \times \text{Standard concentration}$$

2-5-8:Determination of Serum Cholesterol Concentrations:

(187,188)

Principle :

The cholesterol present in the sample originates a colored complex, according to the following reaction:

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenona} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminophenazone} \xrightarrow{\text{POD}} \text{Quinonimine} + 4H_2O$$

The intensity of the color formed was proportional to the cholesterol concentration in the sample.

Reagents:

R1 Buffer	Pipes pH 6.9 Phenol	90 mmol /L 26 mmol/L
R2 Enzymes	Cholesterol e sterase(CHE) Cholesterol oxidase(CHOD) Peroxidase(POD) 4-Aminophenazone(4-AP)	300 U/L 300 U/L 1250U/L 0.4mmol/L
Cholesterol Cal.	Cholesterol aqueous primary standard 200 mg/dL	

Preparation :

Working reagent (WR): The contents were dissolved (→)of one vial R2 enzymes in one bottle of R1 buffer.

The contents were caped and mixed gently.

Procedure :

	Blank	standard	Sample
Working reagent	1 ml	1 ml	1 ml
Standard	…	10 µL	…
Sample	…	…	10 µL

1. Mixed and incubated for 5 minutes at 37˚C or 10 minutes at room temperature.
2. The absorbance (A) of the samples and standard, were read against the blank at wavelength 505nm.

Calculation :

$$\frac{(A)\text{Sample}}{(A)\text{Standard}} \times 200\ (\text{Standarad conc.}) = \text{mg/dL cholesterol in the sample}$$

Conversion factor: mg/dl *0.0258= mmol/L

2-5-9-Determination of Serum Triglyceride Concentration:

Principle: [189,190]

Fossati and Prencipe method associated with Trinder reaction .

Reaction scheme was as follows:

$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{Glycerol + free fatty acids}$$

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

$$\text{Glycerol -3 -Phosphate} + O_2 \xrightarrow{\text{GPO}} \text{DihydroxyacetonePhosphate} + H_2O_2$$

$$H_2O_2 + \text{4-Chlorophenol} + \text{PAP} \xrightarrow{\text{POD}} \text{Quinoneimine (pink)} + H_2O$$

The absorbance of the coloured complex (quinoneimine), proportional to the amount of triglycerides in the specimen, was measured at 500 nm.

Reagents :

№	Contents	Initial Concentrations of Solutions
1	BUFFER Pipes Magnesium chloride Chloro-4-phenol Preservative	 100 mmol/L 9.8 mmol/L 3.5 mmol/L
2	ENZYMES Lipase Perxydase(POD) Glycerol-3-phosphate oxidase (GPO) Glycerol Kinase (GK) 4 –Amino – antipyrine (PAP) Adenosine triphosphate Na (ATP)	 ≥ 1000 IU/L ≥ 1700 IU/L ≥ 3000 IU/L ≥ 660 IU/L 0.5 IU/L 1.3 mmol/L
3	Standard	2.28 mmol/L(200mg/dL)

Procedure:

Pipette into well identified test tubes	Blank	Standard	Assay
Reagent	1 mL	1 mL	1 mL
Dematerialized water	10 µL		
Standard		10 µL	
Specimen			10 µL

Mixed. Let stand for 5 minutes at 37˚C.

The absorbance was recorded at 500 nm (480-520) against reagent blank.

Calculation :

The result was calculated as follows:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standarad)}} \times \text{Standard concentration}$$

2-5-10:Determination of Serum VLDL Concentration:

VLDL was calculated through the following equation:-

VLDL (mmol/L) = Triglyceride/5

2-5-11:Determination of Serum HDL Cholesterol Concentration:

Principle :

Chylomicrons, very low density lipoproteins (VLDL) and low density lipoproteins (LDL) contained in the specimen were precipitated by the addition of phosphotungstic acid in the presence of magnesium ions[191,192]. The supernatant obtained after centrifugation contains high density lipoproteins (HDL).The cholesterol bound to the HDL was determined using the cholesterol RTU reagent.

Content of the kit :

HDL Cholesterol	2*5 ml	Phosphotungstic acid	40 g/l
Precipitant		$MgCl_2,6H_2O$	100 g/l
		Sodium azide	1 g/l
		pH 6.2	

Procedure :

Sample…….500 µl
Precipitating reagent…….50 µl
Mixed. Wait ed10 minutes.
Centrifuge for 15 minutes at 3,750 – 4,160 g.
After centrifugation, the supernatant must be clear.

	Reagent blank	Calibrator	Dosage
Demineralized Water	50 µl	_	_
HDL Cholesterol precipitant	_	50 µl	_
calibrator			
Supernatant	_	_	50 µl
Cholesterol RTU	1 ml	1 ml	1 ml
Mixed. Incubated for 5 mins. At 37˚C Record absorbance at 500 nm against reagent blank.			

Calculation :

$$\frac{\text{A sample}}{\text{A Standared}} \times n$$

mmol/l : n = 1.43

g/l : n = 0.55

2-5-12: Determination of Serum LDL Concentration:

LDL was calculated through the following equation:-

LDL = Total Cholesterol – (HDL + VLDL)

2-5-13:Determination of atherogenic index(AI):

AI was calculated through the following equation:-

Atherogenic Index = LDL / HDL

2-6:-Statistical Analysis:

Statistical analysis was done using the software **SPSS** version 15.0,the results were expressed as mean ± standard deviations (mean ± SD). Tow way ANOVA-test was used to compare parameters in different studied groups. P-values ($P \leq 0.01$) were considered statistically significant.

Person correlation coefficient (r) was used to test the correlation relationship among the different parameters in each patients group.

CHAPTER THREE

RESULTS AND DISCUSSION

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3-1:-Serum High Sensitivity C Reactive Protein Concentrations:

Table (3-1) and Figure (3-1): show a non-significant increase in the concentrations of serum hsCRP in group (AMI) in comparison with group (UA) and there is a significant increase in the concentrations of serum hsCRP in all patients groups in comparison with control group ($P \leq 0.01$).

The result of hsCRP of this study is matched with the result of Ridker P. (2003)[193], Liuzzo *et al.* (1994)[79], and Ferreiros *et al.* (1999)[194] . The levels of hsCRP were above 3µg/ml in all the patients, which is considered abnormally high .However, in all the control group had hs-CRP level less than 3µg/ml.

There is increasingly evidence that inflammation plays an important role in the pathogenesis of atherosclerosis and its complications.

In general, the high hsCRP level is the greater risk of future events becomes. Thus, for healthy persons at risk for coronary artery disease, hsCRP should not be disregarded even it is within the normal range.

High-sensitivity C-reactive protein is an easily measured and widely investigated biomarker of inflammation. It is unclear whether hsCRP itself directly contributes to the pathophysiology of coronary vascular diseases

CVD. supporting data for a role for CRP in atherothrombosis is limited to *in vitro* studies and experiments with animal models [195,196].

A considerable study showed that hsCRP levels were significantly elevated in patients with progressive carotid stenosis and associated with the occurrence of a first future CVD event[197].

Regardless of whether hsCRP plays a causal role in atherothrombosis, this biomarker has been proposed to be useful for improving CVD risk prediction. In this study, the concentrations of hsCRP was increased in patients with CHD but failed to correlate with the severity of coronary disease. This marker might reflect the diffuse atherosclerotic process in the vascular system rather than the degree of localized obstruction from coronary lesions [198].

Table (3-2) shows that all patients were give a positive test for C reactive protein whereas the test was negative for all healthy subjects.

Inflammatory processes play key roles in the initiation and progression of atherosclerosis and its clinical sequelae [65,199]. The relevance of inflammation to atherosclerotic cardiovascular disease (CVD) is such that plasma levels of various inflammatory biomarkers are emerging as important prognostic indicators of future risk of CVD in initially healthy men and women [200].

We found that hsCRP and CRP are useful diagnostic markers for AMI, UA and hsCRP is more sensitive than CRP, but regarding their false positive and negative values, and for decreasing their pitfalls, it is recommended to perform both of them for each case.

Table (3 – 1):- Serum high sensitivity C reactive protein concentrations of (control), (AMI) and (UA) groups

Group	n	hsCRP concentration (mg/L) mean± SD
Control	70	1.33 ± 0.33^{b}
AMI	55	8.11 ± 2.48^{a}
UA	45	7.17 ± 2.65^{a}

* Each value represents mean ± SD values with non-identical superscript (a , b or c …etc.) were considered significantly differences ($P \leq 0.01$).

n: number of subjects.

AMI: Acute Myocardial Infarction.

UA: Unstable Angina/NSTEMI.

SD: Standard Deviation.

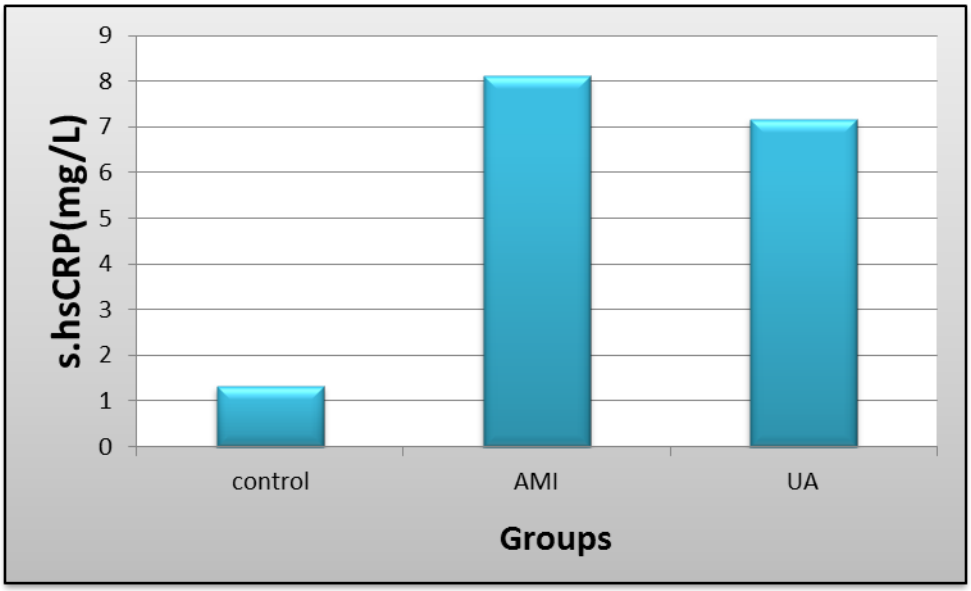


Figure (3-1): Serum hsCRP levels of control and patients groups .

Table (3 – 2):- Serum C reactive protein concentrations of (control), (AMI) and (UA) groups

Group	n	CRP
control	70	Negative
AMI	55	Positive
UA	45	Positive

- Legend as in table (3- 1)

3-2:-Serum Creatine Phospho Kinase Activity:

Table(3-3) and Figure(3-2) show a significant elevation in concentrations of serum CPK in group (UA) in comparison with group (AMI) ($P \leq 0.01$). Also there is a significant increase in the concentrations of serum CPK in the two patients groups in comparison with(control) group ($P \leq 0.01$).

This result compatible with the result of study conducted by (Lansky *et al.* 2010)[(201).] Measurement of CK-MB isoenzyme is the test of choice to confirm the diagnosis of an AMI ,reported that increases in plasma levels usually occur between 3 - 4 hours after the onset of infarction (in the absence of thrombolysis), in general , the total CK activity is often due to myocardial or skeletal muscle injury (Adams *et al*, 1994)[(202).]

Figure(3-3) shows the positive correlation relationship between hsCRP and CPK in (AMI)patients group with correlation coefficient ($r = 0.48$) while in the(UA) patients group, negative correlation relationship between hsCRP and CPK with correlation coefficient ($r = -0.15$). this case due to muscle injury and CPK levels increases are detectable in blood as early as 3-4 hours after the onset of symptoms.

The determination of total creatine kinase (CK) and creatine kinase MB plays a major role in the differential diagnosis and in monitoring of

myocardial infarction patients. Although the methods for determination of these parameters are easy to apply, they are not specific for cardiac muscle damage [(203).]

Approximately 20% of myocardial infarcts are silent, occurring particularly in diabetics, the elderly, Increased serum CK activity is liable to occur, if there is damage to skeletal or cardiac muscles.

The values in the table (3-3) indicate the following:

Possible the patients are NSTEMI or time Acute myocardial infarction in the patients after three days.

Table (3 – 3):- Serum Creatine Phospho Kinase activity of (control), (AMI) and (UA) groups

Group	n	CPK –MB isoenzyme activity (U/L) mean± SD
Control	70	215.44±49.06[c]
AMI	55	674.78±134.76[b]
UA	45	834.24±276.38[a]

- Legend as in table (3- 1)

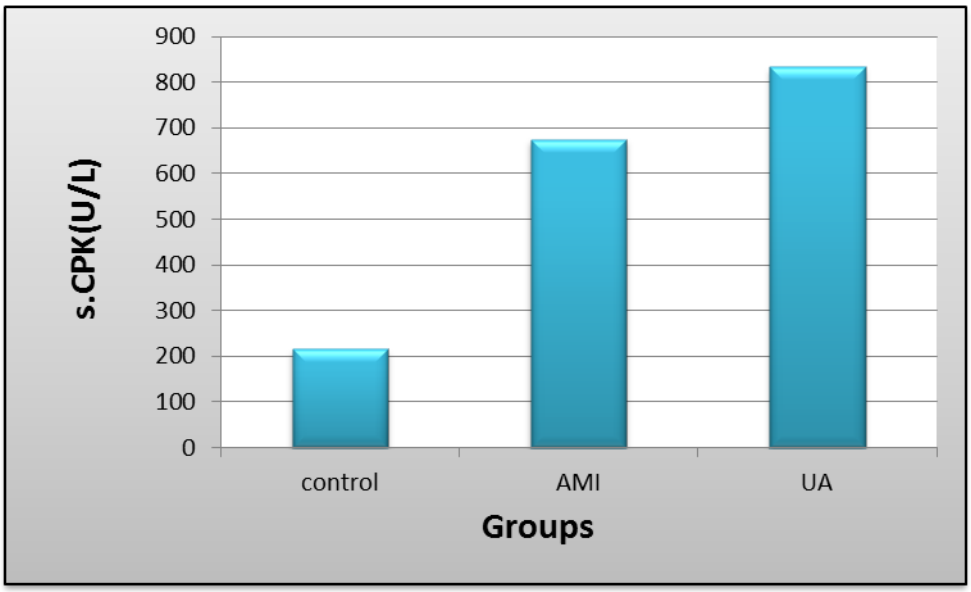


Figure (3-2): Serum CPK levels of control and patients groups .

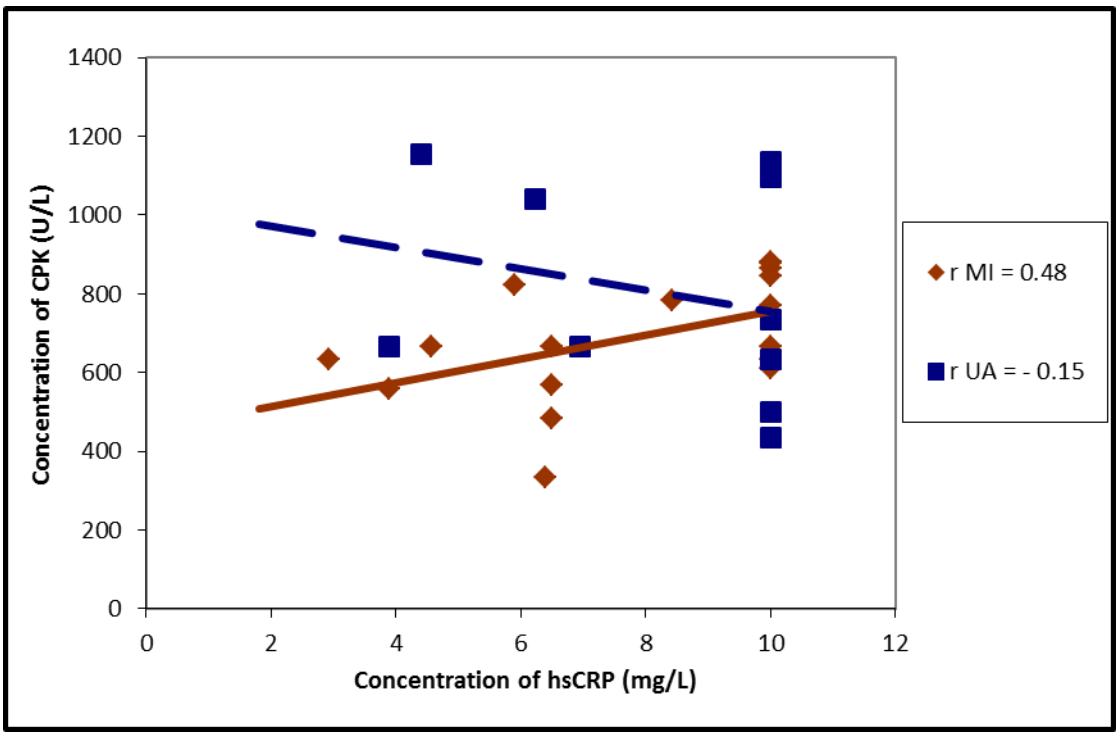


Figure (3-3): Correlation relationship between hsCRP and CPK in patient groups (AMI), (UA)

3-3:-Serum Lactate Dehydrogenase Activity:

Table (3-4) and Figure(3-4) show a non-significant increase in concentrations of serum LDH in group (AMI) in comparison with group (UA) ($P \leq 0.01$) as well as there is a significant increase in concentrations of serum LDH in all patients groups in comparison with control group ($P \leq 0.01$).

Lactate dehydrogenase activities has been recorded in previous study of Burtis *et al.*(1994)[204].It is well known LDH that is used for the diagnosis of acute myocardial infarction Wu *et al.* (1999)[205].

Figure(3-5) shows the positive correlation relationship between hsCRP and LDH in patients groups with correlation coefficient ($r = 0.23$) in group (AMI) and ($r = 0.12$) in (UA) group. The reason due to muscle injury.

LDH is found in almost every tissue, with high activities in skeletal muscle, liver, heart, kidneys, brain, lungs and erythrocytes. LDH exists as a tetramer composed of two different subunits, M (muscle) and H (heart). It is an important enzyme of glucose metabolism. There are five isoenzymes. In the heart, carbohydrate and fatty acid metabolism is relatively constant, metabolites are completely oxidized, and the LDH-1 isoenzyme is predominant. LDH activity starts to increase at 6 to 12 h after the onset of chest pain. Cellular injury can be caused by chemical, microbial and physical agents, but the most important cause is myocardial ischemia. Myocardial ischemia is present whenever the coronary arterial flow fails to provide enough oxygen to meet the demands of the myocardium. The extent of ischemia after the occlusion of a coronary artery depends on the presence of preformed collateral anastomoses. The early release of cardiac markers is influenced by a variety of factors, the most important influence being their intracellular compartmentation.

Sudden induction of myocardial ischemia by the occlusion of a major branch of a coronary artery in the heart sets into motion as series of events that culminates in the death of markedly ischemic myocytes. These events include the cessation of contraction to reduce the energy demand, ECG changes as a result of localized hyperpolarization and
the onset of anaerobic glycolysis. These changes appear rapidly and simultaneously. [206].

Lactate dehydrogenase (LDH), isoenzyme forms of LDH and transaminases. Determination of these cardiac marker enzymes permits a highly sensitive diagnosis of trans mural IHD. It is notable that in such patients the diagnosis of IHD can be confirmed by the clinical symptoms, and changes in the ECG in addition to the enzyme assays.

Table (3 – 4):- Serum Lactate Dehydrogenase activity of (control), (AMI) and (UA) groups

Group	n	LDH activity (IU/L) mean± SD
control	70	220.59±47.65[b]
AMI	55	1694±541.98[a]
UA	45	1584.72±509.67[a]

- Legend as in table (3- 1)

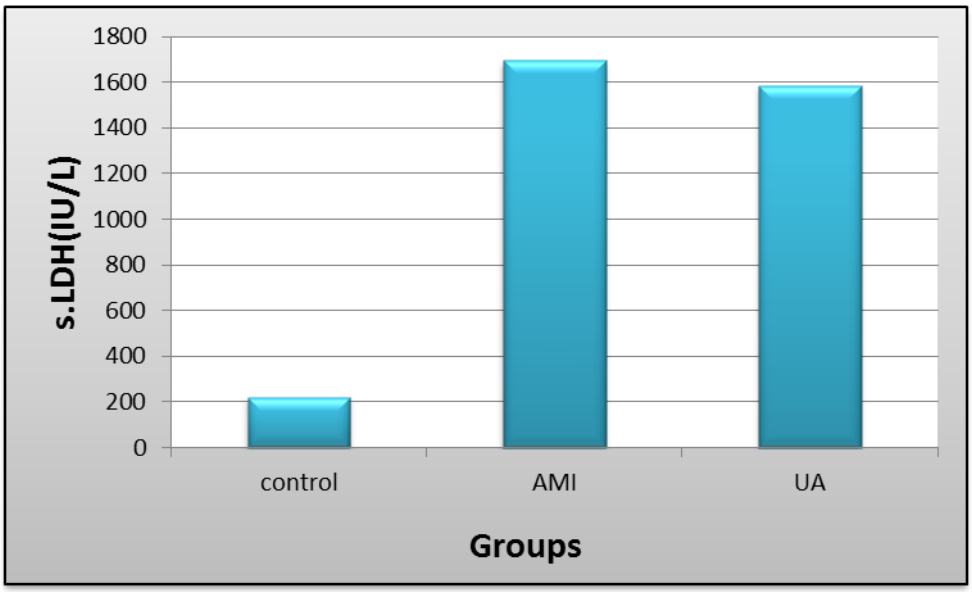


Figure (3-4): Serum LDH levels of control and patients groups .

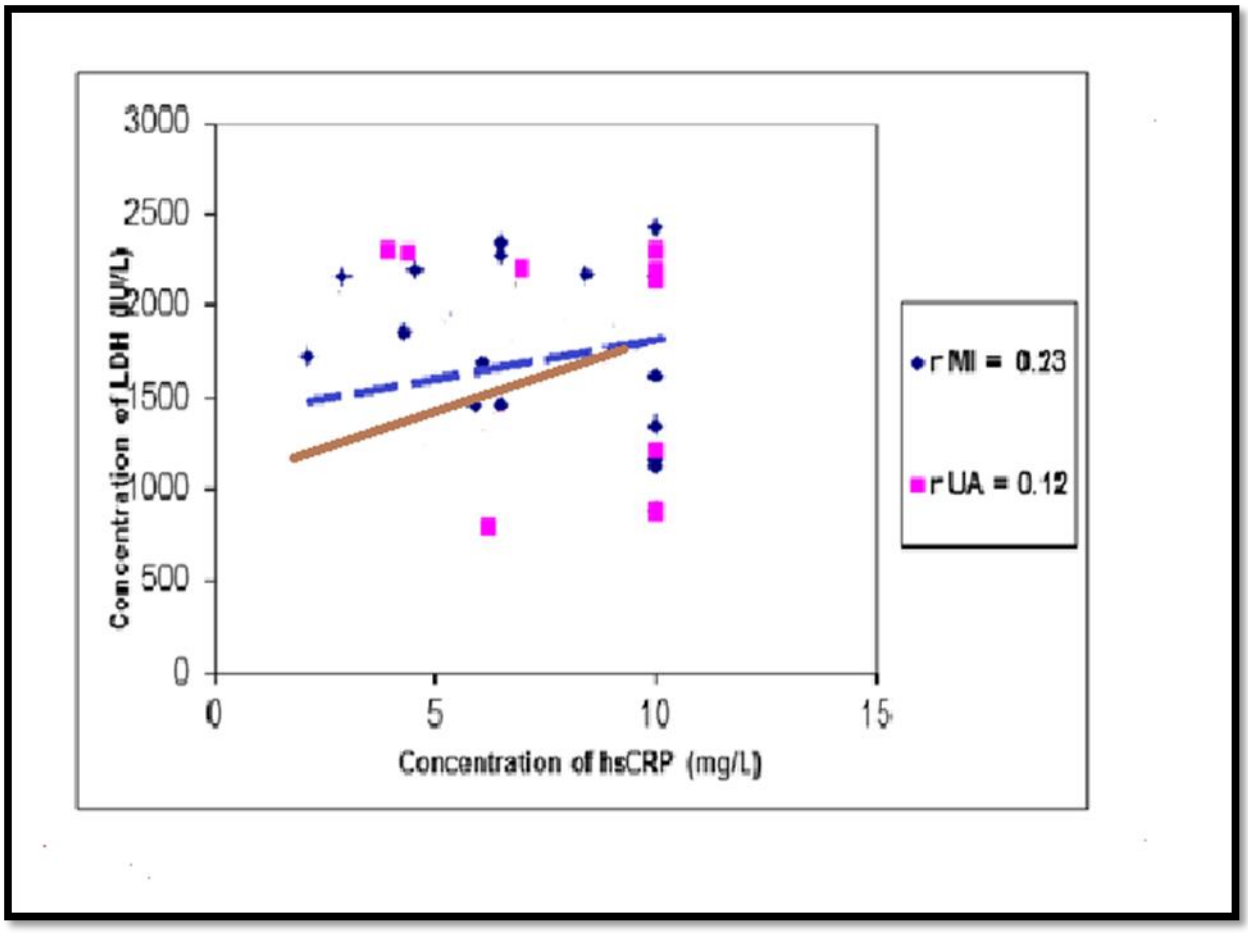


Figure (3-5): Correlation relationship between hsCRP and LDH in patient groups (AMI), (UA)

3-4:- Serum Malondialdehyde Concentrations:

Table (3-5) and Figure (3-6): show that there are no significant differences in serum MDA levels between (AMI) and (UA) groups. While a significant increase in the mentioned parameter levels can be observed in the two patients groups when compared with control group ($P \leq 0.01$).

Generally, in AMI the levels of MDA was significantly higher than those who had no history of AMI. (Puspha *et al.*, 2005)[207]. suggested that reperfusion of the infarct myocardium leads to oxidative stress and there was a highly significant enhancement in the level of MDA.

We observed that increased concentrations of MDA in the circulation of total IHD patients indicating increased lipid peroxidation. Our results are in accordance with previous report (Senthil *et al.*, 2004)[208].

Figure(3-7) shows the positive correlation relationship between hsCRP and MDA in patients groups with correlation coefficient (r=0.26) in group (AMI) and (r = 0.31) in (UA) group. that lipid peroxidation marker (MDA) was higher in patients with IHD, and this may be due to the increasing of oxidative stress in IHD because of free radicals production may ultimately exceed removal resulted in lipid peroxidation.

Lipid peroxidation and antioxidant change and their significance during myocardial injury have provided a new insight in the pathogenesis of heart disease [209]. The increased level of free radical generating system and malondialdehyde (MDA) and lowered levels of free radical scavenging systems seem to have critical role in ischemic heart condition [210]. During times of increased oxygen flux (i.e. exercise). Free radicals have been implicated to play a role in the etiology of cardiovascular disease, the major source of ROS is thought to be the mitochondria of active muscles, but free

radicals are also produced by red blood cells or during inflammatory response. When the antioxidant system is not adapted to excessive production of ROS, oxidative stress initiates. ROS are potent to induce various cellular damage affecting lipids, proteins and nucleic acids. The imbalance between oxidants and antioxidants will affect the normal function of immune cells.

Table (3 – 5):- Serum Malondialdehyde levels of (control), (AMI) and (UA) groups

Group	n	MDA concentration (nmol/mL) mean± SD
control	70	59.33±16.21[b]
AMI	55	124.69±33.20[a]
UA	45	124.51±22.22[a]

- Legend as in table (3- 1)

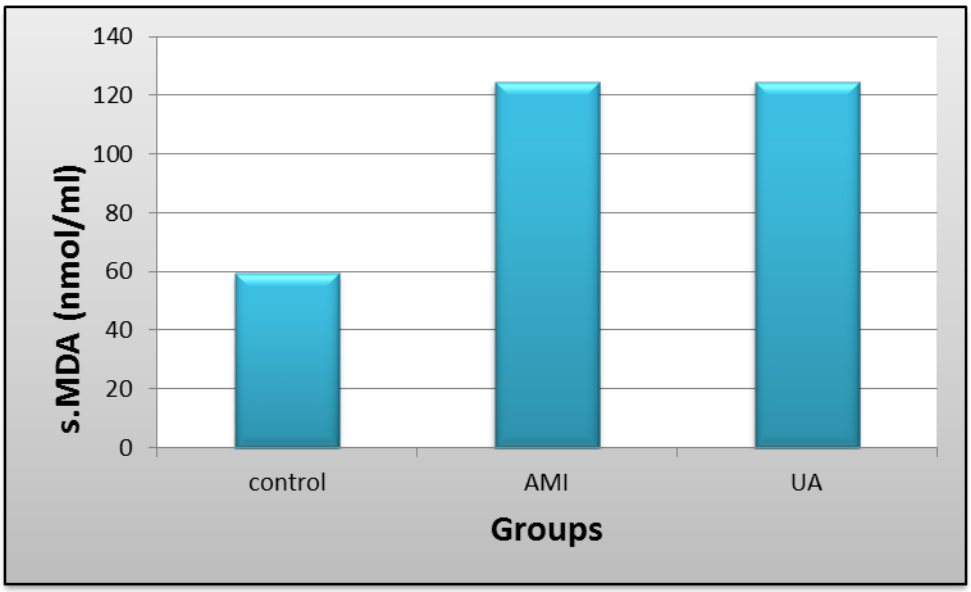


Figure (3-6): Serum MDA levels of control and patients groups .

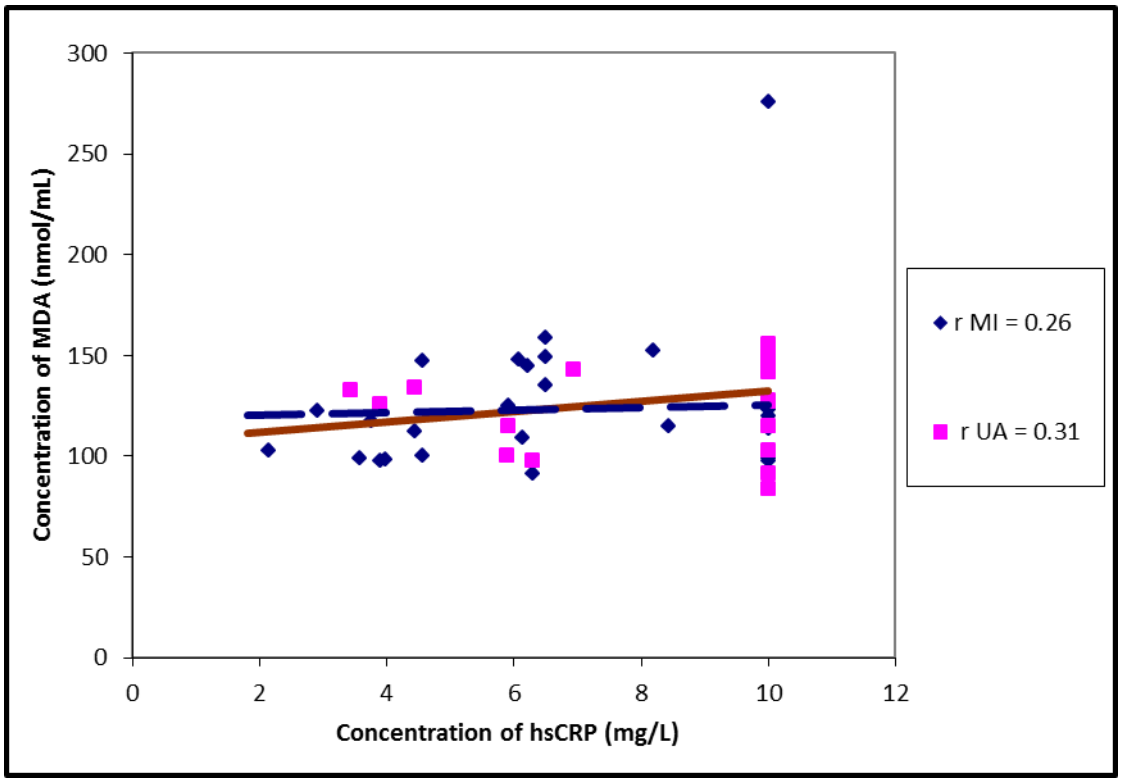


Figure (3-7): Correlation relationship between hsCRP and MDA in patient groups (AMI), (UA)

3-5: Antioxidant system:

3-5-1:-Serum Ceruloplasmin Concentrations:

Table (3-6) and Figure (3-8): show a significant elevation in serum Cp levels in both patient groups in comparison with control group ($P \leq 0.01$). whereas no significant differences in Cp levels can be observed between AMI and UA groups. This finding is matched with the result of (Engstrom *et al*., 2003)[(211)]. that found an increased level of serum ceruloplasmin in IHD patients and suggested that this molecule may act as an oxidative stress indicator, though mechanism remains unclear. Ceruloplasmin is an inflammation-sensitive protein and an acute phase reactant (Hickman and Potter, 2003)[(212).]

Figure(3-9) shows the positive correlation relationship between hsCRP and Cp in patients groups with correlation coefficient (r=0.23) in group (AMI) and (r = 0.58) in (UA) group. ceruloplasmin may have possible role in inflammation. Accordingly ceruloplasmin may be considered as an inflammatory molecule.

ceruloplasmin is an acute phase protein and is synthesized by the liver in response to tissue damage and inflammation [213]. Antioxidant terminates the chain of the reaction by removing free radicals and inhibits other oxidation reaction by oxidizing themselves. The balance between free radicals formation and antioxidant activity is called oxidative stress. When the oxidative stress is unbalanced in favor for free radicals, such as acute and chronic exercises, damage occurs to many cellular membranes such as the heart and skeletal muscles [214].

The effectiveness of the Cp as an anti-oxidation depends on the level of Cu^{+2} in protein, because the Cp works to remove $O_2^{\cdot -}$ the root of superoxide negative through reduced copper atom in the protein.

There are a lot of data which show that free radicals and reactive oxygen species increased cardiovascular risks and also increased damage done on the membrane of living cells.

Table (3 – 6):- Serum Ceruloplasmin concentrations of (control), (AMI) and (UA) groups

Group	n	Cp concentration (g/L) mean± SD
control	70	2.82 ± 0.69^{b}
AMI	55	3.68 ± 0.78^{a}
UA	45	3.93 ± 0.76^{a}

- Legend as in table (3- 1)

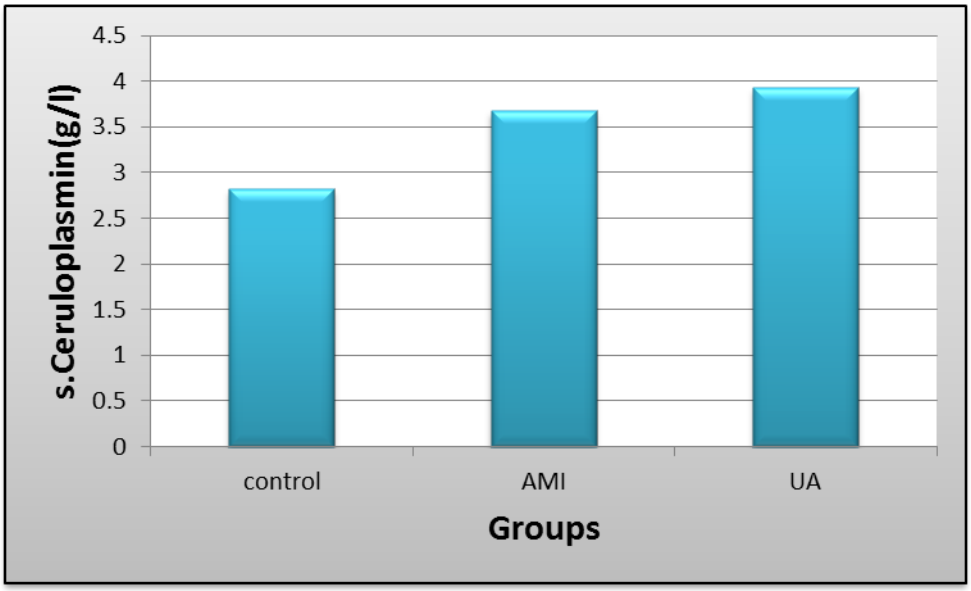


Figure (3-8): Serum Cp levels of control and patients groups.

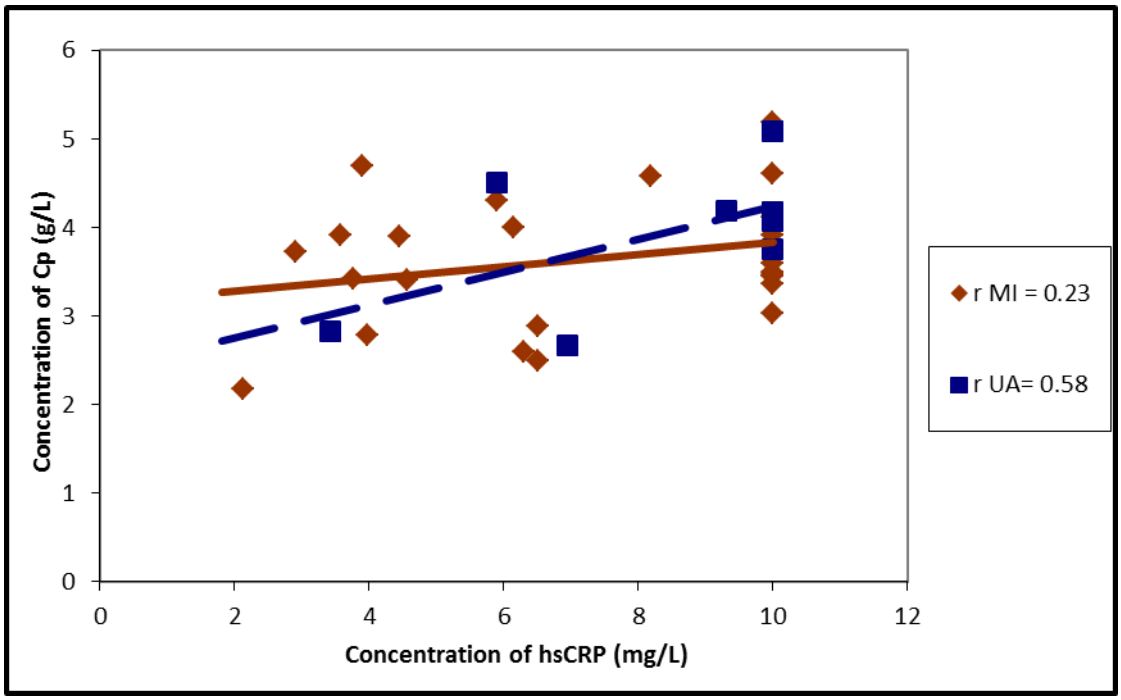


Figure (3-9): Correlation relationship between hsCRP and Cp in patient groups (AMI), (UA)

3-5-2:-Serum Transferrin Concentrations:

The results in table (3-7) and Figure(3-10) show a significant decrease in serum Tf concentration in both patient groups. When compared with control groups ($P \leq 0.01$). whereas no significant differences in concentrations of serum Tf can be observed between AMI and UA groups.

In humans, increased plasma transferrin levels are found in iron deficiency anemia whereas decreased plasma transferrin occurs in conditions resulting in increased iron stores (Morgan, 1983)[(215).] The low plasma transferrin concentration found in humans with increased iron stores may be due to a negative feedback of storage iron levels on transferrin synthesis (Aisen, 1984)[(216).]

The total iron binding capacity was found to be significantly low in these groups reflecting large volume of iron. The high levels of stored iron found in the subjects of coronary heart disease as compared with controls, suggest that there is a cumulative risk of high stored iron in the development of coronary heart disease.

Figure(3-11) shows the negative correlation relationship between hsCRP and Tf in patients groups with correlation coefficient (r= -0.34) in group (AMI) and (r =- 0.31) in (UA) group. Perhaps due in lower Tf concentration in patients with IHD that Tf is one of protective antioxidants that prevent the formation of free radicals. The associated metals and prevent it from interacting with the H_2O_2 to form free radicals, as well as Tf works to remove $O_2^{\cdot-}$ the root of superoxide negative. The Tf working to reduce oxidative stress that occurs in patients with IHD and at least its level in the blood serum.

It is synthesis in the liver and secretes into the plasma. However, this synthesis depends on the intracellular iron concentration, and the synthesis of Tf is regulated in an antagonistic manner[217].

The antioxidant property of transferrin is its ability to bind with iron ions and storage it as a ferritin and prevent the oxidative role of iron which allows to generate free radicals by Fenton and Haber-Weiss reactions[218]. Transferrin, an iron binding protein, transports ferric (Fe^{+3}) iron and store it as ferritin . Specific cell surface receptors for transferrin facilitate and regulate cellular iron uptake. Although iron is required for many biologically important enzymatic reactions, it must be sequestered safely to prevent toxicity. Generally, there is a link which has been established between increased dietary iron intake and increased body iron stores.

Transferrin is one of non- enzymatic that limits the toxicity associated with free radicals. It is known that plasma antioxidant capacity decreases and oxidative/antioxidative balance shifts to the oxidative side in patients with IHD . A reason for increased lipid peroxidation in plasma of patients IHD that found in this study may be a poor enzymatic and non-enzymatic antioxidant defense system including transferrin.

Table (3 – 7):- Serum Transferrin concentrations of (control), (AMI) and (UA) groups

Group	n	Tf concentration (g/L) mean± SD
control	70	10.43±2.57[a]
AMI	55	2.57±0.80[b]
UA	45	2.71±0.89[b]

- Legend as in table (3- 1)

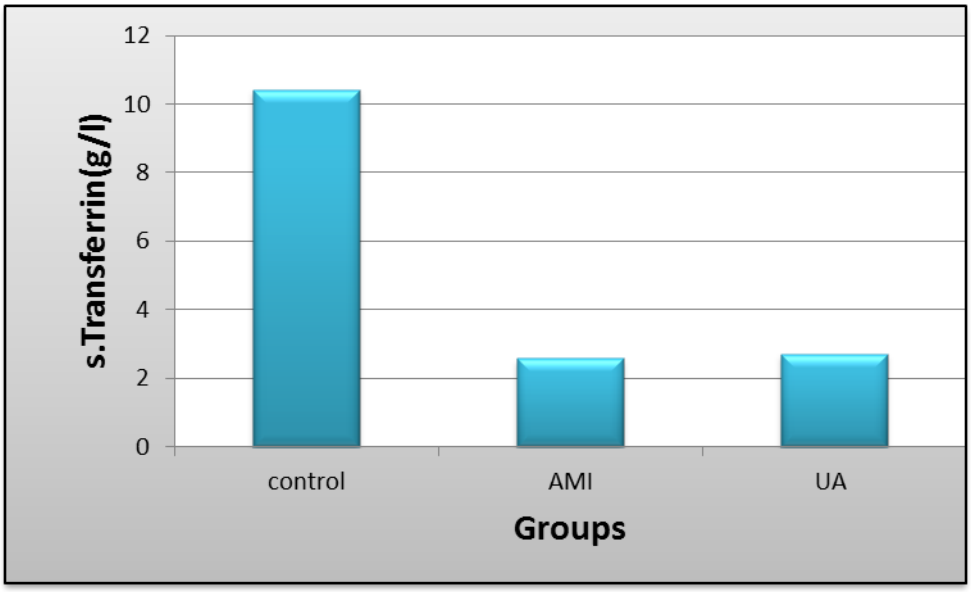


Figure (3-10): Serum Tf levels of control and patients groups .

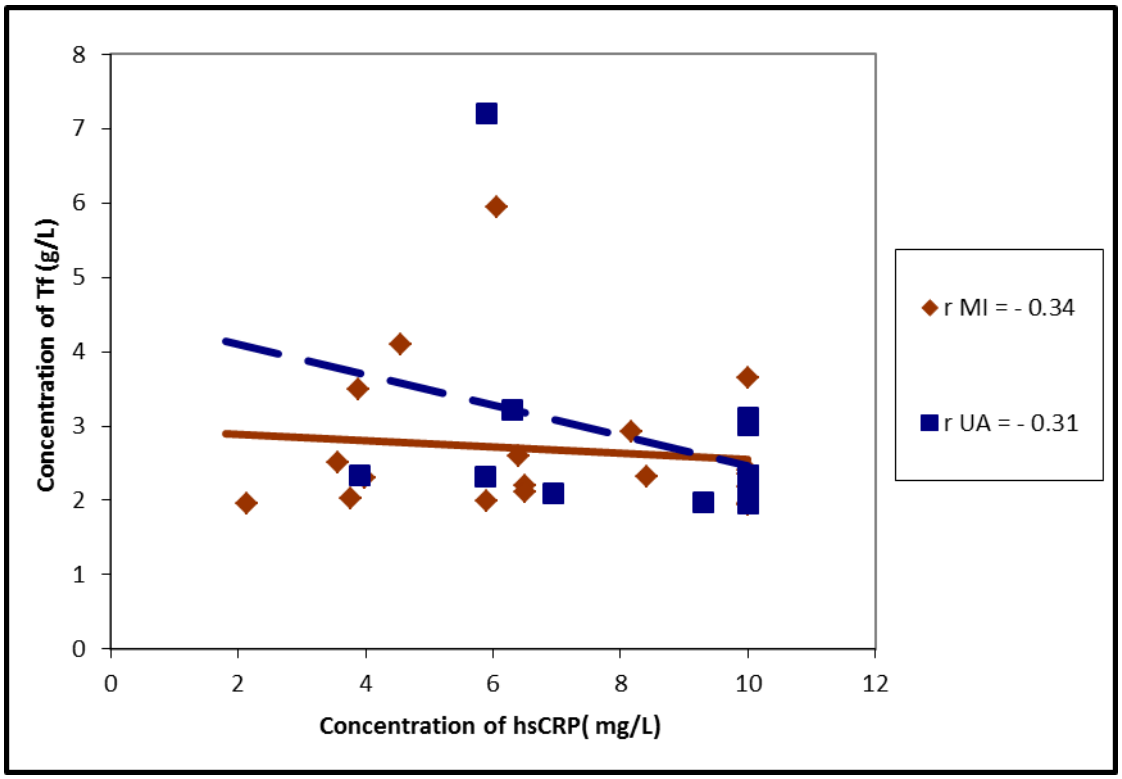


Figure (3-11): Correlation relationship between hsCRP and Tf in patient groups (AMI), (UA)

3-6:Serum Lipid Profile Concentrations:

The results shown in Table (3-8) and Figure(3-12) reveal a highly significant increase of total cholesterol, triglyceride, and VLDL levels than the healthy controls. (P≤0.01). This result is similar to the result of Topsakal, R., *et al*. (2009)[219]. While a highly significant decrease in HDL level in serum of patients with AMI , UA in comparison with that of control group (P≤0.01). These alterations in serum lipoproteins were also confirmed by (Luc *et al*, 2002) [220],(Moselhy and Demerdash 2003)[221], results concerning what they obtained when they measured the concentration of HDL-c in CAD patients after different time periods of CAD occurrence. While highly significant increase in LDL level was noticed in serum of patients with AMI,UA in comparison with that of control group (P≤0.01). This result is similar to the result of (Nigam *et al.*, 2004) [222]. the oxidation of LDL

cholesterol is considered as the most important risk factor for CAD, which plays a central role in atherogenesis**.**

High levels of TCH , LDL and TG and low levels of HDL cause deposition of lipid in arteries thus causing atherosclerosis. So lipid profiles are routinely measured for risk assessment in preventing CAD[(223).]

Lipoproteins represent the form in which most lipids are transported in plasma, the lipoprotein patterns combined with the determination of cholesterol and triglycerides to provide more complete information than to determine these lipids alone.

The lipids and lipoproteins which are central in the metabolism of the body have become increasingly important in clinical practice, primarily because of their association with coronary heart disease [(58).]

Abnormal lipid profile was reported to be an important risk factor for atherosclerosis and ischemic heart disease[(224).] The results of the present study show that there is an association between the incidence, of IHD and abnormal lipid profile.

The results of this study illustrate presence of a positive correlation between hsCRP and each TCH, TG, LDL, and VLDL in both patient groups as shown in figures (3-13), (3-14), (3-16) and (3-17) respectively. Whereas the correlation was negative between hsCRP and HDL in such groups as shown in figure (3-15). With the highest correlation coefficient values. May be that low levels of HDL-c are associated with an increase risk of CHD, and it was confirmed to be a powerful predictor of CHD risk. it is possible to hypothesize that the low HDL-c level observed in this study is associated with an increased cholesterol metabolism in the damaged tissues. the high levels of serum LDL-c are especially atherogenic. Also it should be recalled that atherosclerosis underlies virtually all cases of AMI.

Table (3 – 8):- Serum Lipid Profile concentrations of (control), (AMI) and (UA) groups

Groups	n	TCH mmol/L	TG mmol/L	HDL mmol/L	LDL mmol/L	VLDL mmol/L
control	70	2.56 ± 0.38^{b}	1.02 ± 0.37^{b}	1.28 ± 0.32^{a}	2.04 ± 0.97^{b}	0.21 ± 0.06^{b}
AMI	55	4.72 ± 1.26^{a}	1.56 ± 0.45^{a}	0.86 ± 0.12^{b}	3.57 ± 1.21^{a}	0.37 ± 0.12^{a}
UA	45	4.43 ± 0.86^{a}	1.42 ± 0.40^{a}	0.96 ± 0.19^{b}	3.18 ± 0.85^{a}	0.28 ± 0.10^{a}

- Legend as in table (3- 1)

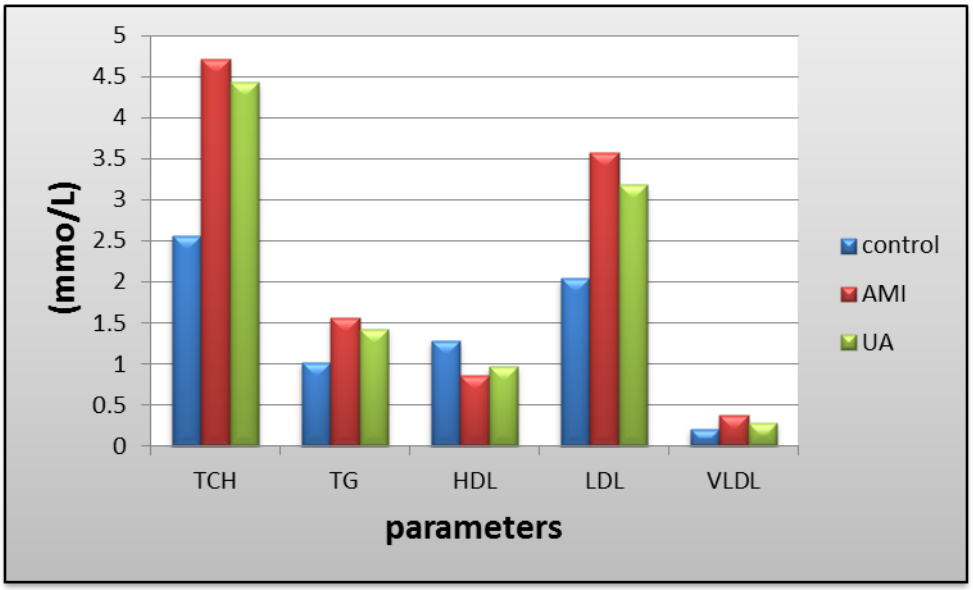


Figure (3-12): Lipid profile levels of control and patient groups

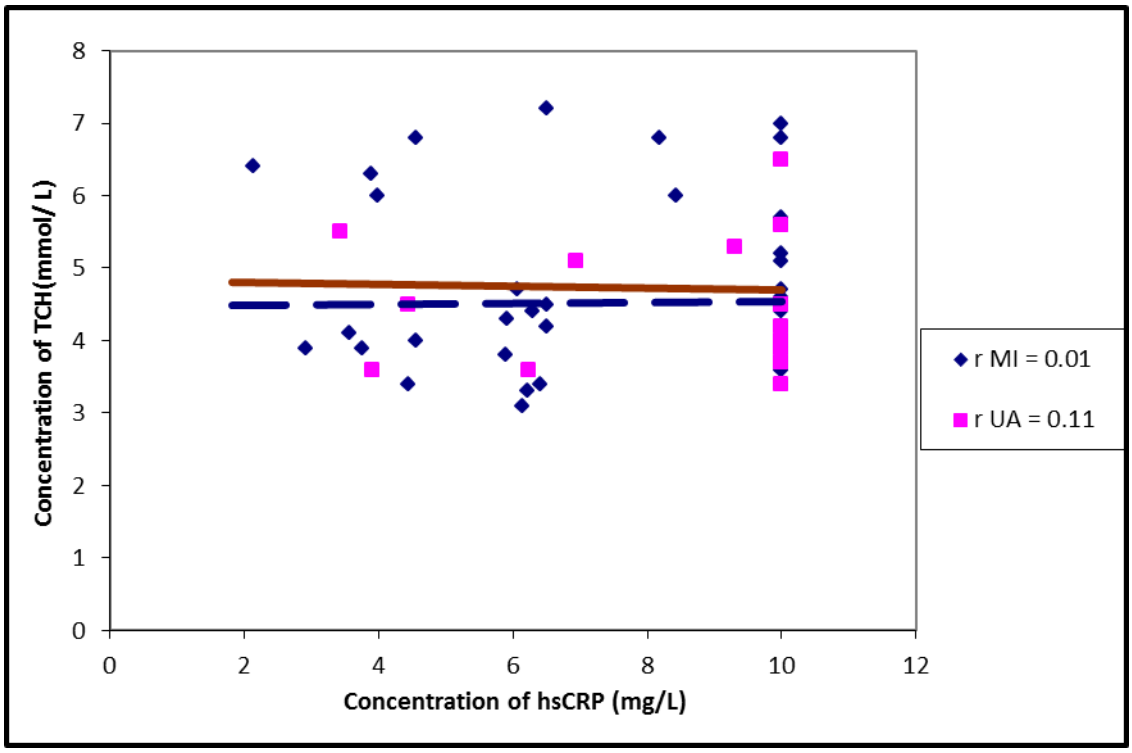


Figure (3-13): Correlation relationship between hsCRP and TCH in patient groups (AMI), (UA)

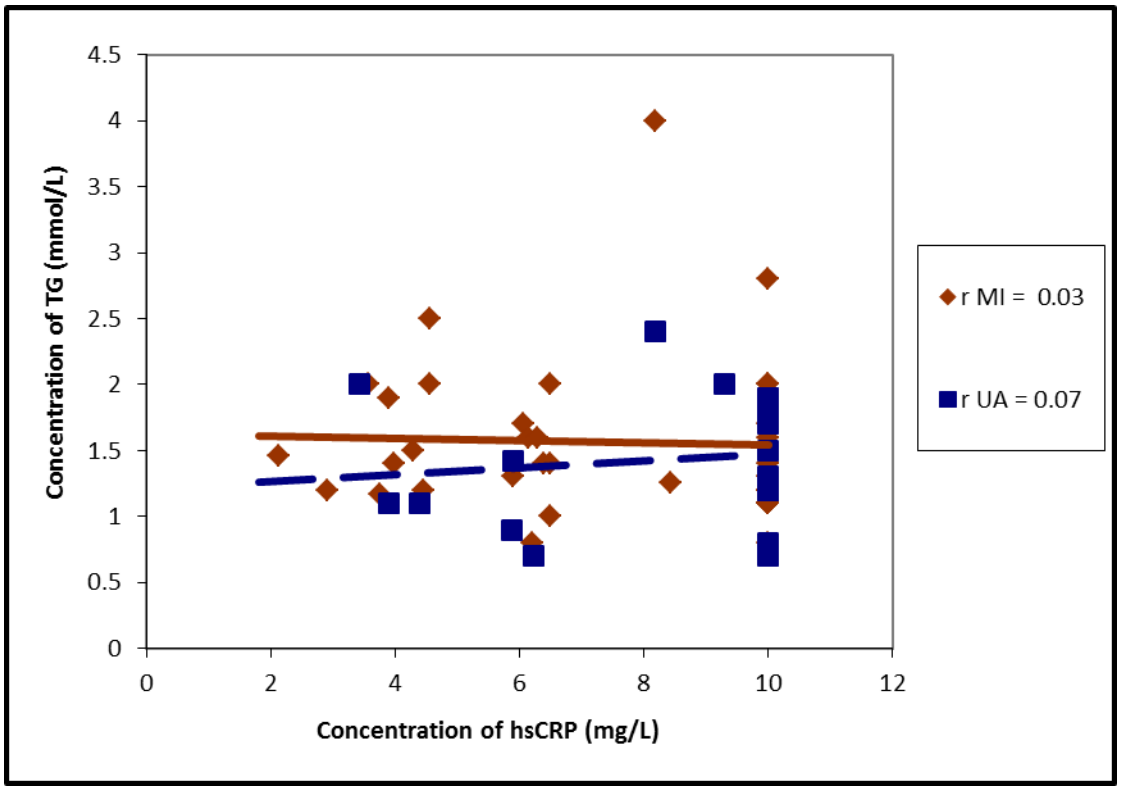


Figure (3-14): Correlation relationship between hsCRP and TG in patient groups (AMI), (UA)

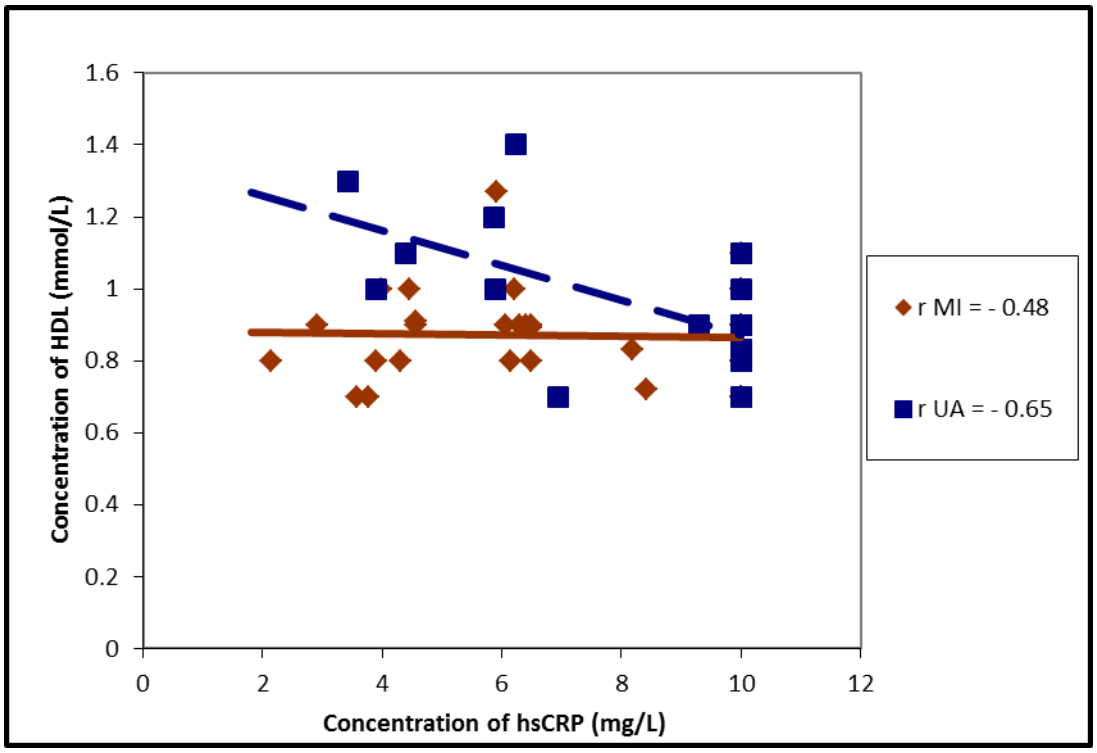


Figure (3-15): Correlation relationship between hsCRP and HDL in patient groups (AMI), (UA)

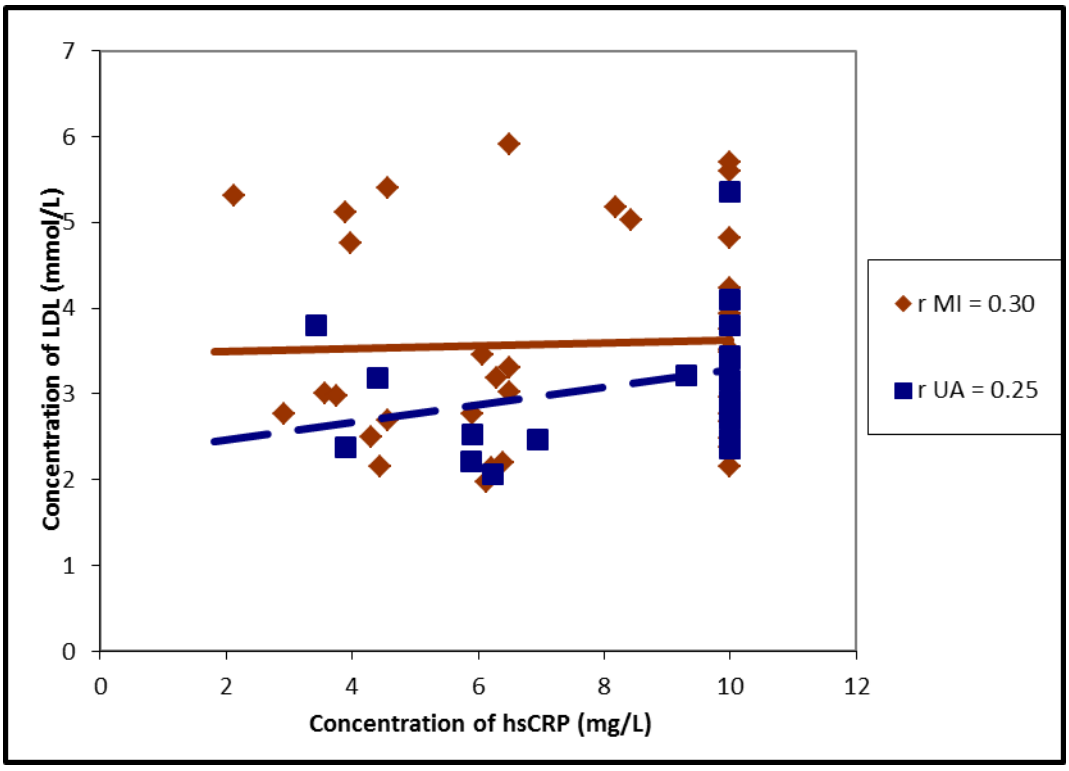


Figure (3-16): Correlation relationship between hsCRP and LDL in patient groups (AMI), (UA)

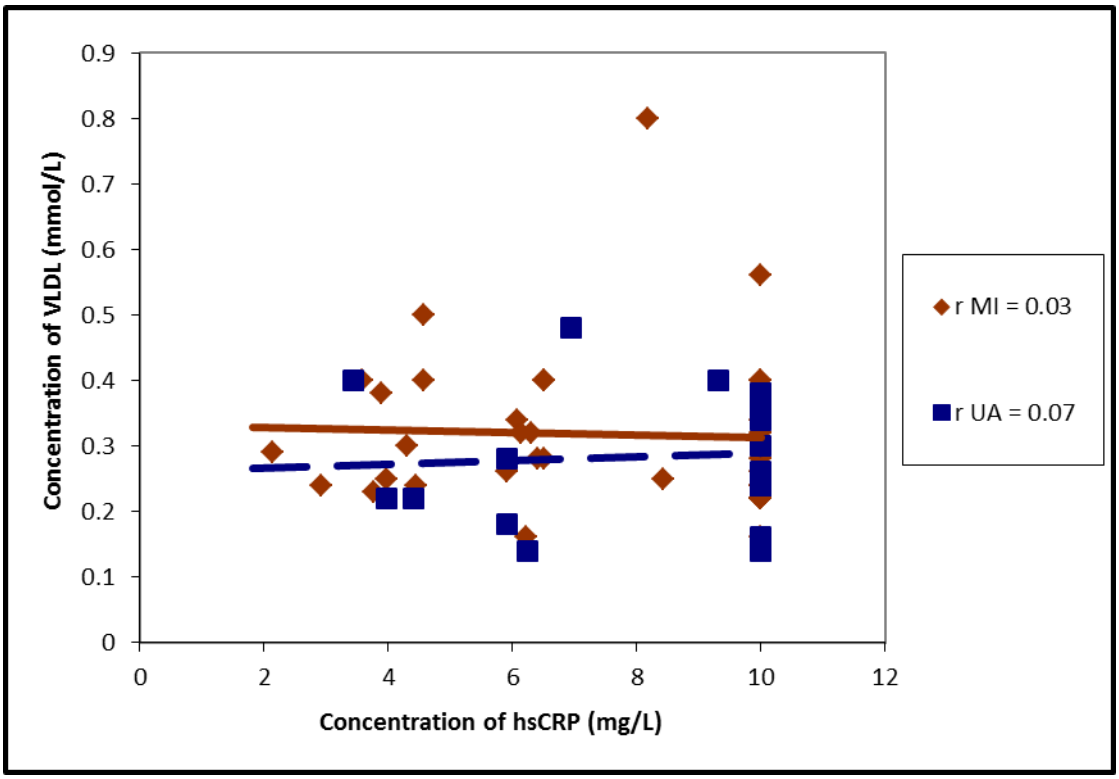


Figure (3-17): Correlation relationship between hsCRP and VLDL in patient groups (AMI), (UA)

3-7:Atherogenic Index Level:

Table (3-9) and figure (3-18) show a significant elevation in serum Ath.Index levels in both patient groups in comparison with control group (P≤0.01). whereas no significant differences in Ath.Index levels can be observed between AMI and UA groups.

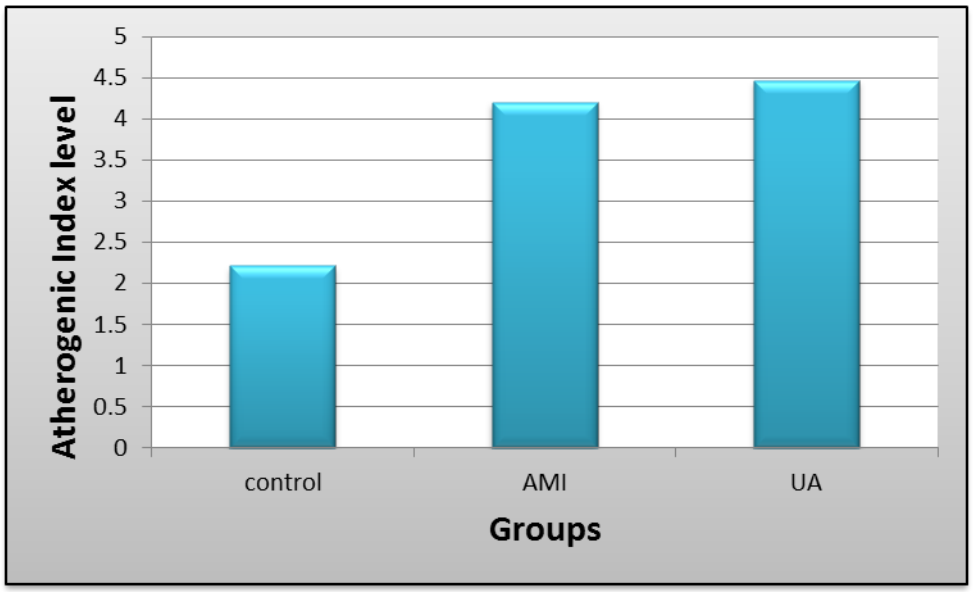
Atherogenic index is powerful indicator of the risk of heart disease: the higher the value, the higher the risk of developing cardiovascular disease [225,226].

Figure (3-19) shows the positive correlation relationship between hsCRP and Atherogenic index in patients groups with correlation coefficient (r=0.41) in group (AMI) and (r = 0.50) in (UA) group. AI was the best predictor of vascular events and AI predicts all-cause mortality. Through this strong relationship can be used hsCRP as additional predictive factor for atherosclerosis diseases and other heart diseases.

Table (3 – 9):-Serum atherogenic index levels of (control), (AMI) and (UA) groups

Group	n	Atherogenic index levels mean ± SD
control	70	2.21 ± 0.88^{b}
AMI	55	4.20 ± 1.56^{a}
UA	45	4.47 ± 1.28^{a}

- Legend as in table (3- 1)



Figure(3-18): Serum atherogenic index levels of (control), (AMI) and (UA) groups

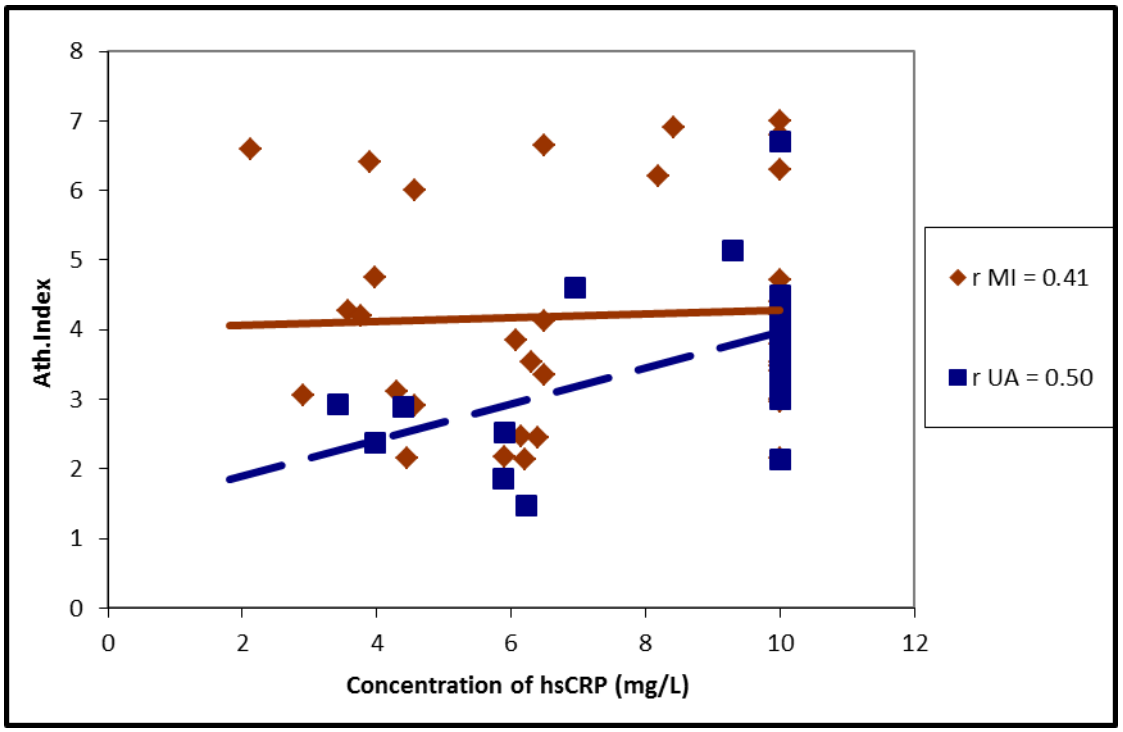


Figure (3-19): Correlation relationship between hsCRP and Ath.Index in patient groups (AMI), (UA)

Conclusions

Form the data presented in this study, we could obtain the following conclusions :-

1-Serum hsCRP may have a significant diagnostic role in Acute coronary syndrome (ACS) (AMI and UA).

2-In both studied diseases , the diagnostic role of myocardial enzymes (CPK and LDH) was confirmed.

3-There is a disorder in antioxidant system in patients with (AMI and UA) according to the levels of ceruloplasmin and transferrin.

4-Lipid peroxidation can be reflected by hsCRP according to the positive correlation between it and MDA.

5-Atherogenic index can be reflected by hsCRP according to the positive correlation between it and AI .

6- The serum levels of hsCRP may be used for risk stratification patients with (ACS) (AMI and UA) and its possible complication .

Future works

More work for the studies are required to examine:-

1- Study the prognostic role of hsCRP in the mentioned diseases.

2- Study the diagnostic role of hsCRP in other heart diseases.

3- Use hsCRP in patients who look healthy but have risk of coronary artery diseases to diagnose IHD before its occurrence.

4-Study the effect of age and sex on hsCRP levels in IHD.

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الخلاصة

تعتبر أمراض القلب الاسكيمية السبب الرئيسي للوفيات في العالم وفي البلدان ذات الدخل المرتفع .وقد تم تصميم الدراسة لتحديد ومقارنة مستويات **بروتين سي الفعال عالي الحساسية(hsCRP)** والانزيمات القلبية **(كرياتين فوسفو كاينيز و لاكتيت ديهايدروجينيز) (CPKوLDH)** وفرط الاكسدة **(المالون ثنائي الالديهايد و السيرلوبلازمين و الترانسفيرين) (MDA و Cp و Tf)** ونمط الدهون **(الكولسترول و الكليسريدات الثلاثية والبروتينات الدهنية عالية الكثافة ،البروتينات الدهنية واطئة الكثافة، البروتينات الدهنية واطئة الكثافة جدا)(TCH و TGو HDL و LDL و VLDL) و (عامل التصلب)(AI)** لمرضى المتلازمة التاجية الحادة والاشخاص الاصحاء .وتقويم معيار بروتين سي الفعال عالي الحساسية في نوعين من المتلازمة التاجية الحادة (احتشاء العضلة القلبية الحاد و الذبحة الصدرية غير المستقرة) للتنبؤ بدورها في مطابقات خطر مرضى المتلازمة التاجية الحادة والاشخاص الاصحاء .

شملت الدراسة (١٧٠) شخص موزعين الى ثلاث مجاميع : (٥٥) مريض باحتشاء العضلة القلبية الحاد (٤٠ رجال و ١٥ نساء)تتراوح اعمارهم (٤٢-٧٨) و (٤٥)مريض بالذبحة الصدرية غير المستقرة (٣٢ رجال و ١٣ نساء) تتراوح اعمارهم (٣٩-٨٠) و (٧٠) شخص من المفترض اصحاء (مجموعة السيطرة) (٤٧ رجال و٢٣ نساء) تتراوح اعمارهم (٣٥-٨٠) .

هدفت الدراسة الى تسليط الضوء على العلاقات الترابطية بين بروتين سي الفعال عالي الحساسية وكل واحد من المعايير التي درست والمذكورة اعلاه .

بينت النتائج ان هناك زيادة معنوية في تراكيز كل من بروتين سي الفعال عالي الحساسية (hsCRP)، اللاكتيت ديهايدروجينيز (LDH) ، مالون ثنائي الديهايد(MDA)، السيرلوبلازمين (Cp)، الكولسترول (TCH) ، الكليسريدات الثلاثية (TG) ، البروتينات الدهنية واطئة الكثافة (LDL)، البروتينات الدهنية واطئة الكثافة جدا(VLDL) وعامل التصلب (AI) في كل مجاميع المرضى مقارنة مع مجموعة السيطرة . وزيادة غير معنوية في تراكيز المعايير المذكورة في مجموعة احتشاء العضلة القلبية مقارنة مع مجموعة الذبحة الصدرية غير المستقرة. كذلك هناك زيادة معنوية في تراكيز الكرياتين فوسفو كاينيز في كل مجاميع المرضى مقارنة مع مجموعة السيطرة وارتفاع معنوي في تراكيز الكرياتين فوسفو كاينيز في مجموعة احتشاء العضلة القلبية مقارنة مع مجموعة الذبحة الصدرية غير المستقرة. بينما نقصان معنوي في تراكيز

(الترانسفيرين (Tf) و البروتينات الدهنية عالية الكثافة (HDL)) في كل مجاميع المرضى مقارنة مع مجموعة السيطرة. ونقصان غير معنوي في تراكيز (الترانسفيرين و البروتينات الدهنية عالية الكثافة) في مجموعة احتشاء العضلة القلبية مقارنة مع مجموعة الذبحة الصدرية غير المستقرة.

كما أظهرت الدراسة وجود علاقة ترابطية موجبة بين (hsCRP) وكل من (MDA, Cp, , TCH,TG, LDL, VLDL, AI LDH,) وعلاقة ترابطية سالبة مع (HDL, Tf).في مرضى احتشاء العضلة القلبية و الذبحة الصدرية غير المستقرة.

بينما العلاقة بين (hsCRP) و(CPK) كانت موجبة في مرضى احتشاء العضلة القلبية وسالبة في مرضى الذبحة الصدرية غير المستقرة.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة ذي قار

كلية العلوم

دراسات سريرية لبروتين سي الفعال عالي الحساسية، فرط الأكسدة ، نمط الدهون و عامل التصلب عند المرضى المصابين بالمتلازمة التاجية الحادة في محافظة ذي قار

رسالة

مقدمة الى مجلس كلية العلوم / جامعة ذي قار

كجزء من متطلبات نيل درجة الماجستير في الكيمياء الحياتية

من قبل

هديل رشيد فرج السنيد

بكالوريوس علوم كيمياء (جامعة ذي قار)

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بأشراف

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