STUDY OF SERUM IRON AND FERRITIN IN PATIENTS WITH ISCHEMIC HEART DISEASES

THESIS

Submitted to The Medical Research Institute,

In partial fulfillment of the requirements for the

Degree of Master of Chemical Pathology

By

Samah Saad Abd El-Aleem El-Sheick

M.B.B.Ch

Alexandria University, 1992

Medical Research Institute

Alexandria University

2001

SUPERVISORS

Prof. Dr. ***Ahmed Mohamad Zaki***

Head of Chemical Pathology Department

Medical Research Institute

Alexandria University

Dr. ***Yousri I. Ahmed Moussa***

Assistant Professor of Chemical Pathology

Medical Research Institute

Alexandria University

Dr. ***Amel Abd El Fattah Kamel***

Lecturer of Chemical Pathology

Medical Research Institute

Alexandria University

Dr. ***Kamal Mahmoud Ahmed***

Assistant Professor of Cardiology and Angiology

Medical Research Institute

Alexandria University

ACKNOWLEDGEMENT

First and before all, thanks to Allah without his help nothing could be reached.

I would like to express my deep and intense feeling of gratitutde to Professor Dr. ***Ahmad Mohamad Zaki***, Head of Chemical Pathology Department, for the guidance, advice and follow up all through this work, and his unlimited efforts in reading and revising this thesis till the final manuscript.

It is my great pleasure to express my gratefulness to Dr. ***Yousri I. Moussa***, Assitant Professor of Chemical Pathology, for the help and experience be offered to me in this study, especially the practical part, may GOD rest him in peace and help his wife and children in their lives.

I am unable to find words sufficiently adequate to express how I am grateful to Dr. ***Amel Abd El Fattah Kamal***, Lecturer of Chemical Pathology, for her deep participation and co-operation with the other supervisors in revising every detail in the thesis and offering me advice in every step of this work.

My great thanks is always to Dr. ***Kamal Mahmoud Ahmed***, Assistant Professor of Cardiology and Angiology, for supplying me

with the materials needed for this study and his noteworthy advices especially from the clinical point of view.

Again words can not describe how grateful I am to my mother for helping me in every possible way and supplying me with an environment suitable for accomplishing this work.

Thanks to all members of the chemical pathology department, that helped me in one way or another in my work, beginning from professors, assistant professors, lecturers, residents, specialists and technicians.

Dedicated to

My Husband

and

My Daughters Shreen & Lora

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AMI	: acute myocardial infarction
Apo A	: Apoprotein A
Apo B	: Apoprotein B
Apo B_{100}	: Apoprotein B_{100}
Apo C	: Apoprotein C
Apo D	: Apoprotein D
Apo E	: Apoprotein E
HCO_3^-	: Bicarbonate
CA	: coronary artery
CHD	: coronary heart disease
CK-MB	: creatine kinase-MB
CU	: copper
DNA	: deoxyribonucleic acid
ECF	: extracellular fluid
ECG	: electrocardiogram
EDTA	: ethylene diamine tetra acetic acid
e^-	: electron
FBG	: fasting blood glucose
Hb	: haemoglobin
Ht	: haematocrit
HCL	: hydrochloric acid
HRP	: horse radish peroxidase
H	: hydrogen
OH	: hydroxyl radical
H_2O_2	: hydrogen peroxide
HDL	: high density lipoprotein
HDL-C	: high density lipoprotein cholesterol
Fe	: iron
IHD	: ischaemic heart disease
LCAT	: lecithin cholesterol acyl transferase
LDL	: low density lipoprotein
LDL-C	: low density lipoprotein cholesterol

LDL-C	: low density lipoprotein cholesterol
MDA	: malondialdehyde
McABs	: monoclonal antibodies
NO	: nitric oxide
NO^+	: nitric oxide radical
O_2	: oxygen
SOD	: superoxide dismutase
TMP	: tetra methyl benzidine
TBA	: thiobarbituric acid
TBARs	: thiobarbituric acid reactive substances
T.Ch.	: total cholesterol
TIBC	: total iron binding capacity
TG	: triglyceride
UV	: ultraviolet
VLDL	: very low density lipoprotein
Zn	: zinc

INTRODUCTION

INTRODUCTION

Ischemic Heart Disease

Ischemic heart disease is defined by the World Health Organization as myocardial impairment due to imbalance between coronary blood flow and myocardial requirements caused by changes in the coronary circulation.[1]

The imbalance is usually related to either an absolute reduction in coronary blood flow or inability to increase coronary blood flow relative to the needs of the heart and most often due to atherosclerotic obstruction of large coronary artery.[2]

Coronary atherosclerosis is most prominent in the main stem of the coronary arteries, particularly in the segments closest to the ostia of the coronary vessel.[3]

Risk factors for atherosclerosis:

Risk factors are certain biological variations or habits of life which can be observed more commonly in coronary patients than in general population.[4] They have traditionally been divided into two categories:[3,5]

a- Modifiable risk factors:

(hyperlipidaemia, hypertension, smoking, diabetes, overweight, physical inactivity and psychological factors).

b- Non modifiable risk factors:

(age, gender and hereditary factors).

However more recent data indicate clustering of several metabolic and familial factors that are often related to each other.[5]

1- Hyperlipidemia:

The association of various lipid parameters with the progression of coronary disease is well established and hyperlipidaemia is a known positive risk factor for coronary arteriosclerosis.[6]

2- Hypertension:

Coronary disease is now the commonest sequela of hypertension. The incidence of cardiovascular disease and the factors that predispose to it including hyperlipidaemia, hyperuricaemia, glucose intolerance and obesity are more common in hypertensive persons than in the general population.[7]

When the blood pressure exceeds 160 mmHg systolic and 95 mmHg diastolic in middle-aged men the risk is five times greater than in normotensive men.[3]

3- Cigarette smoking:

Smokers are at high risk of developing atherosclerosis and cardiac ischemic disease. Cigarette smoke contains vast amounts of both carbon- and oxygen-centered free radicals, which can directly initiate and propagate the process of lipid peroxidation.[8]

Recently, several studies have implicated oxidative modification of LDL by cigarette smoke as a possible explanation.[9-11]

4- Glucose intolerance and diabetes mellitus:

Diabetics show at least twofold increase in the incidence of myocardial infarction, compared with nondiabetics. Younger diabetics have a marked increase in the risk of ischemic heart disease, and diabetic women appear to be even more prone than diabetic men.[3]

5- Age:

"Aging theory of atherosclerosis" supposes that atherosclerosis is an inevitable consequence of ageing and therefore an irreversible process.[12]

6- Sex:

Male gender remains one of the most powerful factors at all levels of risk.[3] Epidemiological studies has shown that women loose their protection against CHD after menopause. This cardioprotection in premenopausal women has generally been contributed to endogenous estrogen[13] which might influence the development of atherosclerosis by their effect on the distribution or nature of the plasma lipoproteins.[3]

7- Genetic factors:

They are critical in atherosclerosis. The best example of this is the increased incidence of atherosclerosis in individuals with homozygous familial hypercholesterolaemia and familial combined hyperlipidaemia.

Other risk factors such as hypertension and diabetes mellitus can also be inherited and protective factors as increased HDL may also be inherited.[3]

8- Obesity:

When body weight is greater than twenty percent above the normal, there is increased risk of ischaemic heart disease. Obesity is generally associated with hypertriglyceridaemia, hypercholesterolaemia, glucose intolerance and hypertension.[3]

9- Physical inactivity:

Sedentary individuals are more susceptible to atherosclerosis and to sudden death than individuals who maintain an active lifestyle. Increased physical activity may elevate HDL levels.[3]

Pathology

Almost all myocardial infarctions result from coronary atherosclerosis. Generally with superimposed coronary thrombosis.[14]

Role of acute plaque change:

Slowly occurring high-grade stenosis of epicardial coronaries may progress to complete occlusion but do not usually precipitate AMI. Probably because of the development of a rich collateral network over time. However, during the natural evolution of atherosclerotic plaques, especially those that are lipid-laden, an abrupt and catastrophic transition may occur characterized by plaque rupture and exposure of substances that promote platelet activation and thrombosis generation. The resultant thrombus interrupts blood flow and leads to an imbalance between oxygen supply and demand and, if this imbalance is severe and persistent, it will lead to myocardial necrosis.[15-21]

Composition of plaques:

At autopsy, the atherosclerotic plaque of patients who died of MI is composed primarily of fibrous tissue of varying density and cellularity with superimposed thrombus.[22-24]

Calcium, lipid-laden foam cells, and extracellular lipid each constitute 5 to 10 percent of the remaining area.[22]

The atherosclerotic plaques that are associated with thrombosis and a total occlusion, located in infarct-related vessels, are generally

more complex and irregular than those in vessels not associated with MI.[25]

Histological studies of these lesions often reveal plaque rupture or fissuring.[16,25,26]

Angiographic morphology suggestive of plaque rupture has been identified in the majority of stenosis associated with AMI or abrupt onset of unstable angina.[27]

Platelet-rich thrombi are often associated with the surface of the most advanced atherosclerotic lesions, called complicated plaque, which are characterized by fibrocalcific degeneration, deposition of lipid, calcium, fibrous tissue, necrotic debris, extravasated blood, and a fibrous cap.[25]

Impaired endothelial cell function may contribute to atherogenesis through release of growth factors. Luminal narrowing may potentiate platelet activation through augmentation of chear forces.[25]

Young persons with coronary thrombotic events have been described as having a genetic polymorphism in glycoprotein IIb/IIIa, possibly altering platelet-fibrinogen interactions. This observation raises the possibility of screening for patients at increased risk of coronary thrombosis in the event of plaque rupture.[28]

Plaque fissuring and rupture:

The process of plaque fissuring is an area of intense investigation and is likely to be multifactorial in nature.[20]

Lippy has summarized the evidence suggesting that T lymphocytes in human atheroma elaborate the cytokine interferon gamma (IFN-γ) that markedly inhibits the ability of vascular smooth muscle cells to form interstitial collagen in vulnerable regions of the fibrous cap over an atherosclerotic plaque.[25]

Furthermore, in atherosclerotic plaques prone to rupture there is an increased rate of formation of metalloproteinase enzymes such as collagenase, gelatinase, and stromelysin that degrade components of the protective interstitial matrix.[21,25,29]

These proteinases may be elaborated by activated macrophages and mast cells that have been shown to accumulate in high concentration at the site of atheromatous erosions and plaque rupture in patients who died of AMI.[19,30,31]

Reactive oxygen species and free radicals are produced continuously in the body as a result of normal metabolic processes. Free radicals can play a beneficial role in fighting off infection. For example, phagocytes in the blood attack and destroy disease-causing microbes through a mechanism involving free radicals.[32] However, free radicals can also damage complex molecules such as proteins[33] and lipids[34] or even DNA.[35]

Free radicals have been lately associated with diseases such as cancer, heart disease, rheumatoid arthritis and it is speculated that numerous diseases in both adult and neonatal medicine are associated with free radicals and their damaging effect.[36]

Definition:

A free radical can be defined as a chemical species possessing one or more unpaired electron(s) in their outer orbital. It can also be considered as a fragment of a molecule.[37] The unpaired electron and the radical nature of a species are conventionally indicated by writing it with a heavy superscript dot.

Mechanism of formation of free radicals:[38]

1- By the homolytic cleavage of a covalent bond of a normal molecule with each fragment retaining one of the paired electrons.

$$X : Y \longrightarrow X + Y$$

Homolytic fission generally requires high energy input resulting from either high temperature, UV light or ionizing radiation

2- By the loss of a single electron from a normal molecule.

$$\text{e.g.} \quad NO - e^- \longrightarrow NO^+$$

3- By the addition of a single electron to a normal molecule.

$$\text{e.g.} \quad O_3 + e^- \longrightarrow O_2^- \text{ (superoxide free radical anion)}$$

Electron transfer is a far more common process in biological system than is homolytic fission.

$$A + e^- \longrightarrow A^-$$

As a consequence of having an unpaired electron in their outer orbital, free radicals have an increased reactivity with other molecules.

Oxygen free radicals and reactive oxygen species:

One of the most important molecules in free radicals biochemistry is oxygen. Electrons are distributed in oxygen in such a way that two of the electrons are unpaired.

Reduction of oxygen by the transfer to it of a single electron will produce the superoxide free radical anion.

$$O_2 + e^- \longrightarrow O_2^-$$

Reduction of oxygen with 2 electrons would yield hydrogen peroxide

$$O_2 + 2e^- + 2H \longrightarrow H_2O_2$$

2 superoxide molecules can react together with hydrogen to form hydrogen peroxide and oxygen.

$$2O_2^- + 2H \longrightarrow H_2O_2 + O_2$$

Hydrogen peroxide is not a free radical, but it is an important compound in free radical biochemistry because it can easily breakdown

in the presence of transition metal ions, to produce the most reactive and damaging of the oxygen free radicals, the hydroxyl radical.

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

(Fe-catalyzed HaberWeiss reaction or fenton reaction)

The superoxide can react directly with hydrogen peroxide:

$$O_2^- + H_2O_2 \longrightarrow OH + OH^- + O_2$$

(noncatalysed Haber Weiss reaction)

The iron (or copper) catalysed reaction is considered to be dependant on superoxide.

$$O_2^- + Fe^{3+} \longrightarrow Fe^{2+} + O_2$$

$$O_2^- + Cu^{2+} \longrightarrow Cu^+ + O_2$$

The auto-oxidation of reduced transition metals can also generate superoxide.

$$Fe^{2+} + O_2 \longrightarrow Fe^{3+} + O_2^-$$

$$Cu^+ + O_2 \longrightarrow Cu^{2+} + O_2^-$$

Thus the reactions of the transition metal ions with oxygen can be considered reversible redox reactions and are extremely important in the promotion of the free radical reactions.[39]

Thus "the keyplayers" in the biochemistry of oxygen free radicals are oxygen itself, superoxide, hydrogen peroxide, transition metal ions and the hydroxy radical, the first 4 of which conspire by a variety of reactions to generate the last.[36,38,40]

Superoxide:

It is not a damaging species, its main significance is probably as a source of hydrogen peroxide and as a reductant of transition metal ions.

Hydrogen peroxide:

It is a source of hydroxyl radical in the presence of reactive transition metal ions.[41]

The hydroxyl radical:

It is an extremely reactive oxidising radical that will react with most biomolecules. It has an extremely short half-life, but is capable of causing great damage within a small radius of its site of production.

Oxygen free radicals are not the only important free radicals in biochemistry, although they are often the initial species formed. Carbon centered radicals $R^{\cdot}$ arise from the attack of an oxidising radical on a biological molecule such as lipid, nucleic acid, carbohydrate and protein.[37]

Sulphur atoms can be the centre for free radicals RS e.g. in the oxidation of glutathione.[38]

Nitric oxide free radicals $NO^{\cdot}$ produced by phagocytes and vascular endothelial cells acts on smooth muscle cells in vessel walls to produce relaxation.[42,43]

Production of free radicals in cells:

The major source of free radicals in cells is electron leakage from electron transport chains, such as those in mitochondria and in endoplasmic reticulum, to molecular oxygen generating superoxide.[42]

Other enzymes can also produce superoxide or hydrogen peroxide, such as flavin oxidases located in peroxisomes. Another source of superoxide in the cells is the autoxidation of certain compounds including ascorbic acid (vitamin C), thiols (glutathione, cysteine), adrenaline and flavin coenzymes.[37]

An important enzymatic oxygen radical generating system is the hypoxanthine-xanthine oxidase system. It is considered as a cytosolic source for free radical formation.[36]

$$O_2 + \text{Hypoxanthine} \xrightarrow{\text{xanthine oxidase}} O_2^{\cdot-} + \text{urate}$$

Prostaglandin production through the arachidonic acid cascade involves the formation of free radicals.[44]

Assay for free radicals:

Thiobarbituric acid reactive substances, (TBARs) assay is the most popular and easiest method used as an indicator of lipid

peroxidation, TBA react with malondialdehyde (MDA), heating is required then after extraction into an organic solvent fluorimetric measurement is applied.[45] In uncharacterized systems it is usual to refer to the assay of TBARs (TBA reactive substances) as the test is not specific for MAD. Direct assessment of free MAD is most reliably done by HPLC.[45]

Low-density lipoprotein and atherogenesis:

Clinical and experimental studies of various kinds have firmly established that elevated plasma concentrations of low-density lipoprotein (LDL) are associated with accelerated atherogenesis.[46-48]

The cholesterol that accumulates in atherosclerotic lesions originates primarily in plasma lipoproteins including LDL.[49] Most foam cells arise from circulating monocytes that have taken up residence beneath the vascular endothelium,[50,51] although some are derived from medial smooth-muscle cells.[51,52] According to response-to-injury theory of atherogenesis, endothelial cell injury is the basic mechanism for initiation and maintenance of atherosclerosis and is followed by atherosclerotic lesions.[53,54]

The arterial uptake, giving rise to foam cells and fatty streaks must be by pathways independent of the LDL receptor, as lesions rich in macrophage-derived foam cells develop even in patients and animals deficient in functional LDL receptors.[51,55,56]

Cell-induced oxidation of LDL:

When LDL was incubated with cultured endothelial cells for 12 to 18 hours, it underwent a striking series of physical and chemical changes and most important, that the resulting endothelial cell-modified form of LDL was taken up by cultured macrophages 3 to 10 times more rapidly than native LDL.[57,58]

It has been shown that monocytes and macrophages can themselves effect similar modification in LDL.[59,60]

Cell-induced oxidative modification can be mimicked by simply incubating LDL in a serum-free medium in the presence of a sufficiently high concentration of copper or iron.[61,62] Whatever the details of the initiating step may be, once the LDL contains fatty acid lipid peroxides, there follows (especially in the presence of metal ions) a rapid propagation that amplifies dramatically the number of free radicals and leads to extensive fragmentation of the fatty acid chains.[63]

Some workers suggest that shorter-chain aldehydes (e.g., malondialdehyde and 4-hydroxynonenal) are generated, and some of these can attach covalently to apoprotein B.[63]

Cytotoxicity of oxidized LDL and its possible role in atherogenesis

It is shown by some workers that oxidized LDL is highly cytotoxic, but native LDL is not.

Because certain cell types, including endothelial cells, can themselves oxidatively modify LDL, the incubation of native LDL with such cells can lead to cytotoxicity; this can be prevented by the addition of antioxidants.[64]

Considerable experimental evidence implicates endothelial injury as an important factor in atherogenesis.[65] However, frank denudation of the endothelium does not appear to occur before the development of the fatty-streak lesion. Still, the cytotoxicity of oxidized LDL can conceivably induce functional changes in the endothelial cell that favor the penetration of circulating monocytes or favor the movement of LDL into the subendothelial space and thus accelerate the formation of fatty streak.[66]

The role of apolipoproteins in the development of atherosclerosis

APO A:

APO A is the major protein component of HDL. HDL functions as a circulating acceptor of cholesterol from peripheral cells. Under normal conditions there is a net transport of cholesterol from peripheral tissue cells to the site of excretion and metabolism in the liver.[67] Most plasma cholesterol is in the esterified form, where as efflux from peripheral cells is in the form of free cholesterol.[68]

The major factor catalyzing cholesterol ester synthesis in plasma is lecithin cholesterol acyl transferase (LCAT). LCAT reactivity is dependent upon apolipoprotein A-I and the reaction is inhibited by apolipoprotein A-II.[69,70] Efflux of cholesterol is in part catalyzed by a lipoprotein containing apoprotein A-I unassociated with apoprotein A-II. The LCAT is present in plasma in the form of a complex with a minor apoprotein, apoprotein D.[71]

APO B:

Apo B-100 forms the main protein mass of LDL. LDL is the principal carrier of cholesterol in plasma and it is the most atherogenic class of cholesterol carrying lipoproteins. The level of plasma LDL is regulated by the LDL receptor, a cell surface glycoprotein, that specifically binds lipoproteins that contain apoprotein B-100.[72]

The Apo B containing LDL interacts with the receptors initiating a series of intracellular events including internalization, lysosomal degradation and regulation of cholesterol metabolism.[73]

The liver has a wide capacity to Apo B production, which may or may not be associated with hypercholesterolaemia or hypertriglyceridaemia.[74,75]

Patients with primary over-production of ApoB appear to be at an increased risk for coronary heart disease.[76]

APO C:

APO C plays an important role in the metabolism of triglyceride rich lipoproteins.[77] APO C-II is a specific Co-factor essential for triglyceride hydrolysis by extrahepatic lipoprotein lipase and its deficiency leads to hypertriglyceridemia.[78] However, APO C-III acts as specific inhibitor of such an enzyme.[69]

APO D:

The APO D may participate in the activation of the LCAT system and may be a specific carrier of the lysolecithin formed after LCAT has acted on HDL.[69]

APO E:

A subfraction of HDL_2 (unique in containing the E apoprotein) sometimes called HDL with APO E has got a specific antiatherogenic

function. It is produced in response to tissue overload with cholesterol to mediate its removal from tissues.[73]

It was found that lipoproteins containing the APO E such as the chylomicron remnants are cleared with much greater efficiency than the lipoproteins containing APO B such as LDL.[79]

The APO E seems to play an important role in the binding of lipoproteins to cell surface receptors.[69,80]

Iron Metabolism and transport

Iron participates in numerous metabolic pathways in all cells and organisms. Its ability to exist in Fe^{+3} and Fe^{+2} states underlines its importance in oxygen and electron transport system concerned with cellular energy production. The liver is intimately involved in many aspects of iron metabolism as a result of the presence of numerous iron dependent metabolic functions.[81]

In acidic solutions, iron ions are surrounded by six water molecules, and have the forms of $Fe(H_2O)_6^{+2}$ and $Fe(H_2O)_6^{+3}$. At physiological pH Fe^{+2} is reasonably soluble; whereas the Fe^{+3} is almost insoluble.[82]

Iron compounds

The iron content of adult humans is approximately 40 mg/kg in women and 50 mg/kg in men.[82] This may be subdivided into:

Transport iron:

As transferrin which is glycoprotein about 30% saturated with iron and other forms of transport iron as, lactoferrin, haptoglobin and albumin.

Also iron in plasma can be complexed with some chemicals such as citrate, ascorbate and amino acids.

Storage iron:

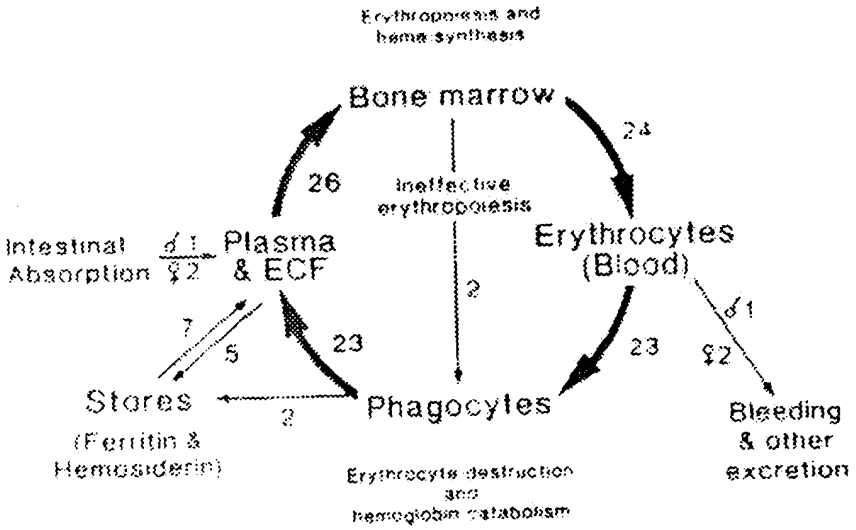
as ferritin and haemosiderin.

Functional iron proteins:

Functional iron containing proteins can be divided into those involved in oxygen transport e.g.: hemoglobin and myoglobin and the heme and nonheme iron containing enzymes as oxidase, peroxidase and catalase.[83]

Pathways of iron metabolism

Iron is tightly conserved in a nearly closed system in which each iron atom cycles repeatedly from plasma and extracellular fluid (ECF) to the bone marrow, where it is incorporated into hemoglobin. Then it travels to the peripheral blood, where it remains within erythrocyte in the blood for 4 months. It then travels to phagocytes of the reticuloendothelial system, where senescent erythrocytes are engulfed and destroyed, hemoglobin is digested, and iron is released to plasma, where the cycle continues. With each cycle, a small proportion of iron is transferred to storage sites, where it is incorporated into ferritin or hemosidrin, a small proportion of storage iron is released to plasma, a small proportion is lost in urine, sweat, feces or in blood, and an equivalent small amount of iron is absorbed from the intestinal tract. In addition a small proportion (about 10%) of newly formed erythrocytes normally is destroyed within the bone marrow and its iron released bypassing the circulating blood part of the cycle (ineffective erythropoiesis).



Pathways of iron metabolism.[84]

The numbers indicate the approximate amount of iron (in mg) that enters and leaves each of these iron compartments every day in healthy adults who do not have bleeding and other blood disorders.[84]

Transport iron:

Transferrin:

It is a glycoprotein (containing about 6% carbohydrate). It consists of single polypeptide chain of molecular weight 79,570 containing 679 amino acids, organised into two domains of approximately equal size.

Each domaine contains one binding site for iron in ferric state.

(C terminal domain and N terminal domain).[85]

The iron in the transferrin is in the ferric state. Each molecule of transferrin can bind two ferric atoms with simultaneous binding of one HCO_3^- for each ferric atom bound.[86]

The stability of the iron-transferrin complex is dependant on the oxidation state of the iron, the presence of the anion, and the confirmation of protein as well as the pH of the solution. Therefore iron can be released from transferrin by reduction of iron, removal of the anion, deformation of the protein (when interacts with receptor) or acidification of the solution.[87]

Iron uptake by the cells:

Transferrin receptor:

The function of transferrin receptors is to mediate the cellular uptake of iron bound transferrin. In most types of cells, this is achieved by receptor-mediated endocytosis; but in some cells, such as hepatocytes iron possibly can be released from transferrin while it is on the plasma membrane of the cell.[88]

Receptor mediated endocytosis of transferrin:

After binding to its receptor at the cell surface, the transferrin receptor complex is endocytosed into the cell. This is followed by acidification to pH 5.5-6.5. The iron is then released from transferrin and transported to the cytosol.

The apotransferrin remains bound to the receptor and recycled to the cell membrane by exocytosis. At the extracellular pH,

apotransferrin, has poor affinity to its receptor. Apotransferrin is thus displaced from the receptor by iron containing transferrin and the cycle is repeated.[88]

Once iron has passed through the plasma and then the endosomal membrane, it is transported to intra cellular sites, to be incorporated into heme, ferritin and other non heme iron protein.[82]

Other forms of transport iron:

Several proteins, that bind iron or iron containing compounds are present in the plasma and play subsidiary roles in the iron transport especially in abnormal conditions such as; iron overload, hemolytic states and inflammatory conditions. Those proteins include, ferritin, lactoferrin, hemopexin, haptoglobin and albumin.[89]

Storage iron:

Ferritin:

Ferritin is water soluble, iron binding protein with wide distribution in nature. The ferritin molecule is approximately spherical. It consists of an outer protein coat and inner iron rich electron-dense core. The core of the ferritin molecule can contain 4000 iron atoms in the form of ferric hydroxyphosphate.[90]

Iron is believed to enter the ferritin core in the ferrous state through hydrophilic channels, it is then oxidised into ferric ions and stored into the molecule. When cells are in the state of positive iron

balance, synthesis of ferritin is stimulated and the iron is deposited as ferritin in a non toxic storage form.[91]

When the cell goes into negative iron balance, iron is released from ferritin to enter the metabolic pathways or leave the cell for use elsewhere in the organism.[92,93]

Hemosidrin:

Cellular storage iron is also present in water insoluble form called hemosidrin. It consists of iron oxyhydroxy cores and the protein of hemosidrin reacts with antiferritin antibodies but the iron: protein ratio is higher in hemosiderin than in ferritin.[94]

Hemosidrin is probably formed by the partial digestion of ferritin by lysosomal enzymes. In normal subjects, storage iron is two thirds in the form of ferritin and one third hemosidrin, but in iron overload the hemosidrin increases considerably.[94]

Oxidative stress:

It has been defined as a disturbance in the equilibrium status of pro-oxidant/antioxidant systems in intact cells.

This definition of oxidative stress implies that cells have intact pro-oxidant/antioxidant systems that function continuously to generate and to detoxify oxidants during normal aerobic metabolism. When additional oxidative events occur, the pro-oxidant systems may outbalance the antioxidant, resulting in oxidative damage to lipids,

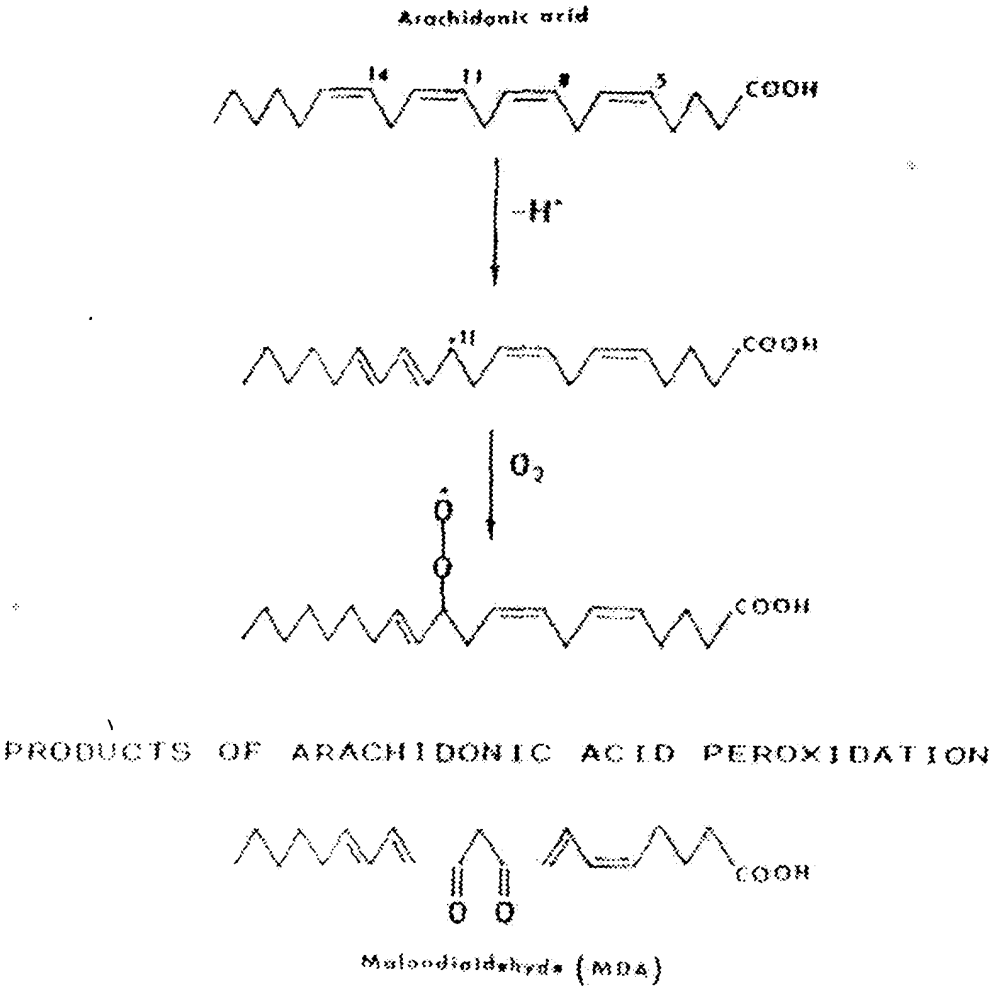
proteins, carbohydrates, and nucleic acids. Oxidative stress may also induce a rapid alteration in the antioxidant systems by depleting cellular stores of antioxidant materials such as glutathione and vitamin E. A disturbance in pro-oxidant/antioxidant systems results from a myriad of different oxidative challenges, including radiation, xenobiotic metabolism of environmental pollutants and other materials, and challenges to the immune system in human disease.[95]

Effect of oxidants on macromolecules:

Lipids:

Peroxidation of cellular lipid results in a variety of deleterious effects on important membrane functions. Most peroxidized lipid occurs as a result of oxidative stress in intact cells, but some peroxidized lipid in the diet may be directly incorporated into cell structures.[96]

The reaction of this process shown in figure below.



Reactions of lipid peroxidation.[96]

The products of lipid peroxidation are easily detected in blood plasma and have been used as a measure of oxidative stress. The most commonly measured product is malondialdhyde.[96]

In addition the unsaturated aldhydes produced from these reactions have been implicated in the modification of cellular proteins and other

materials. Vitamin E is particularly effective as an antioxidant in lipid peroxidizing systems.[97]

Cellular antioxidants:

The most effective antioxidant in oxidative stress depends on the specific molecules causing the stress (e.g., superoxide anion, lipid peroxides, iron generated hydroxyl radical) and the cellular or extracellular location of the source of these molecule. As an example, damage to a cell membrane occurs from both internally and externally generated oxidative stress. This damage is most effectively prevented by vitamin E, which reacts with peroxyl and hydroxyl radicals; by carotenoids, which react with singlet oxygen; and possibly by membrane bound proteins. The chain-breaking anti-oxidant function of vitamin E in membranes results form its close association with polyunsaturated components of the membrane.[98]

Vitamin E radical can be reduced by cytoplasmic vitamin C and glutathione, or by membrane-bound quinols.

Similarly, when oxidative stress occurs in plasma, a variety of different antioxidants participate in the response. Many plasma proteins are affected by the process, causing either irreversible or reversible loss of functional protein activity.[99]

Enzymes:

Superoxide dismutase is one of the most important enzymes that function as cellular antioxidants. It is present in cell cytoplasm (copper-

zinc enzyme) and in mitochondria (manganese enzyme) in order to maintain a low concentration of superoxide anion.[100]

$$2\ O_2^{\cdot-} + 2\ H^+ \xrightarrow{\text{superoxide dismutase}} O_2 + H_2O_2$$

The absence of this enzyme is lethal, but increasing its concentration in cells may not increase antioxidant protection. On the other hand, it may produce hydrogen peroxide at a rate that makes it more toxic to cells in oxidative stress.

Superoxide dismutase is increased by specific redox-sensitive genes in cells under continued stress.[101]

An extracellular form of superoxide dismutase, different from the intracellular forms of the enzymes, occurs in plasma, lymph and synovial fluid.[102]

The extracellular enzymes may function at cell surfaces. Catalase is a heme protein. It is usually found in peroxisomes. In cells such as erythrocytes, which don't contain these organelles, it is a cytoplasmic enzyme. Catalase provides a protective role that is similar to that of glutathione peroxidase in most cells.[103]

$$2\ H_2O_2 \xrightarrow{\text{Catalase}} O_2 + 2H_2O$$

Superoxide dismutase and catalase provide a rapid means of equilibrating and detoxifying superoxide anion and hydrogen peroxide in cells.

In addition, both enzymes have considerable use as pharmacologic agents to decrease the effect of oxygen radicals in human disease.

There has been interest in these enzymes for prevention of reperfusion injury.[104]

Plasma antioxidants:

Human plasma contains little catalase, superoxide dismutase, glutathione, or glutathione peroxidase; the antioxidant properties of this important fluid reside primarily in a number of small molecules and protein constituents.

In studies using both artificially generated oxidants and natural oxidants such as cigarette smoke, ascorbate is among the first compounds that become oxidized in stress.[105,106]

The antioxidant proteins of plasma that are most important include ceruloplasmin, albumin, transferrin, haptoglobin and hemopexin. The first three proteins may sequester iron and copper ions in forms that prevent their participation in reactions that generate the aggressive hydroxyl radical. Haptoglobin and hemopexin bind free heme, a source of iron that can participate in lipid oxidation reactions

(oxidative stress also affects plasma lipid particles such as low-density lipoproteins (LDL)).[106] The proteins and lipids in LDLs are good targets for oxidation, and the oxidized forms of LDL are now strongly implicated in formation of fatty lesions (atheromas) in artery walls. The apoprotein B component of these particles is fragmented by oxidation.

A variety of lipid peroxidation products, including adducts of lipid and apoprotein B, are produced. LDL particles contain a significant amount of vitamin E and carotinoids that may serve as primary antioxidants.[98]

Iron in human atheroma and LDL oxidation by macrophages following erythrophagocytosis

The oxidative modification of low density lipoprotein (LDL) within vessel walls, as well as its uptake by macrophages, are considered important steps in the development of the early atheromatous lesion.[107]

It has been shown that LDL modification is due to free-radical chain reactions and that both copper and iron in reduced forms are able to induce such processes.[108]

Macrophages after engulfing aged or damaged erythrocytes, or other cellular material, may contain secondary lysosomes with catalytically active low-molecular weight iron complexes. If such macrophages are located in the arterial wall in the close vicinity of LDL

particles, these cells might exocytose iron with reduction of ferric iron into its catalytically active ferrous form. An iron dependent oxidative process may thus be initiated, leading to oxidation and conversion of the LDL to a form that promotes lipid accumulation in macrophages.[109]

Iron could promote the modification of LDL by initiating lipid peroxidation or by promoting the breakdown of lipid hydroperoxides.[110,111]

The mechanisms by which iron initiates lipid peroxidation are far from clear. It could catalyse the formation of hydroxyl radicals by a Haber Weiss reaction or Fenton reaction.[112]

$$H_2O_2 + Fe^{2+} \longrightarrow oH^{\cdot} + oH^{-} + Fe^{3+}$$

or could possibly form preferryl ions (Fe^{2+}-O_2) or a ferrous-dioxygen-ferric complex.

These species could then initiate lipid peroxidation. Both Fe^{2+} and soluble Fe^{3+} complexes promote the conversion of lipid hydroperoxides to free radicals (lipid alkoxy and peroxyl radicals respectively).[113]

These radicals can stimulate the chain reaction of lipid peroxidation or can fragment by complex reactions to form a variety of compounds including aldehydes, which may react with apolipoprotein B-100.[114,115]

Iron has been shown to bind to phospholipids in liposomes and microsomes and this raises the interesting possibility that iron-mediated free-radical generation may actually occur in the surface of LDL particles.[116]

Aim of The Work

AIM OF THE WORK

The aim of the present work is to study serum iron and ferritin in patients with ischemic heart disease in a trial to evaluate their possible role in coronary atherosclerosis.

MATERIAL & METHODS

MATERIAL AND METHODS

This study included:

- Thirty patients with ischemic heart disease.

They were subdivided into two subgroups:

A- Fifteen with stable angina.

B- Fifteen with unstable angina pectoris.

Unstable angina pectoris was diagnosed if:

1- Angina worsened in term of frequency, intensity, ease of provocation.

2- Rest pains, nocturnal and prolonged episodes.

3- Recent onset of typical angina within one month, provided that no ECG or enzymatic evidence of infarction.

- Ten apparently healthy subjects as control group.

All the subjects were of comparable age and sex, and clinically free.

METHODS

To all subjects included in the present study, the following were done:

1- Thorough history taking:

With special stress on presenting complaint including:

- Chest pain; its type, duration and radiation.
- Other symptoms as dyspnea and orthopnea.
- Autonomic symptoms as nausea, vomiting and excessive sweating.

2- Full clinical examination.

3- Standard twelve lead ECG.

4- Laboratory investigations.

Sample collection:

- All blood samples were taken in the morning after at least 12 hours fasting.
- Subjects were seated for 20 minutes before withdrawal of blood.
- About 10 ml of blood were withdrawn from the anticubital vein with minimal stasis using disposable plastic syringes.
- The ten ml were divided as follows:

  * 2ml into a tube containing EDTA for complete blood picture. Then this blood were centrifuged and separated plasma was used for assay of TBARs.

* A serum sample was obtained. The separated serum was used for assay of fasting blood sugar, kidney function, lipid survay test, tests to asses iron status and superoxide dismutase.

- An early morning specimen of urine was collected for every subject and examined as soon as possible the same morning.

Laboratory investigations included in the present study:

- Urine analysis.[117]
- Complete blood picture.[118]

Fasting blood glucose level: (bioMérieux kits)[119]

It was determined by using an enzymatic method based on the following reactions:

1st step: (specific for glucose)

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{Gluconic acid} + H_2O_2 \text{ (hydrogen peroxide)}$$

2nd step: (not specific for glucose)

$$H_2O_2 + \text{reduced chromogen} \xrightarrow{\text{peroxidase}} \text{oxidized (rose coloured product) chromogen} + H_2O$$

The rose coloured product which is proportionate to the concentration of glucose in the sample (T) was measured spectrophotometrically at λ505 nm.

The concentration of glucose was determined after comparison with a standard glucose solution of known concentration (mg/dl) (S) similarly treated and glucose was calculated as follows:

$$\text{mg glucose/dl} = T/S \times Cs \text{ (mg/dl)}$$

$$\text{mmol glucose/L} = \text{mg/dl} \times 0.055$$

- Serum urea: (bioMerieux kits)[120]

It was determined by an ultraviolet kinetic method (urease/glutamate dehydrogenase coupled system) according to the following reactions.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$2NH_4 + 2\alpha\text{ketoglutarate} + 2NADH + H \xrightarrow{\text{Glutamate dehydrogenase}} 2\text{-L glutamate} + 2H_2O + 2NAD$$

The sample without deproteinization was taken, mixed well with the reagents and the variation in optical density per minute was read at 30 and at 90 seconds (Δ) at λ 340 nm (T).

A standard urea solution of a known concentration (mg/dl) (S) was similarly treated and urea was *calculated as follows*:

$$\text{mg urea/dl} = \Delta T/\Delta S \text{ X Cs (mg/dl)}$$

$$\text{mmol urea/L} = \text{mg/dl X } 0.166$$

Serum creatinine: (bioMerieux kits)[121]

It was determined kinetically without deproteinization. The complex formed by creatinine in the sample and picric acid in an alkaline medium (sodium hydroxide) was measured at an interval of 1 min at λ 500 nm. A standard creatinine of a known concentration (mg/dl) was similarly treated.

The difference in optical density at 20 and 80 seconds (Δ) was used to determine the creatinine in the sample (T) and standard (S) according to the following equation:

$$\text{mg creatinine/dl} = \Delta T/\Delta S \text{ X Cs (mg/dl)}$$

$$\text{mmol creatinine/L} = \text{mg/dl X } 0.0884$$

Total serum cholesterol:[122]

It was determined enzymatically without deproteinization according to the following reactions:

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

$$\text{Cholesterol} \xrightarrow{\text{cholesterol oxidase}} \text{cholest-4-ene-3-one} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{4-amino antipyrene} \xrightarrow{\text{peroxidase}} \text{quinonemine} + 4H_2O$$

The rose coloured chromogen which was proportionate to cholesterol concentration in the sample (T) was measured at λ 505 nm, and compared to the colour of a standard of a known cholesterol concentration (mg/dl) similarly treated (S).

The concentration of cholesterol was obtained by the following equation:

$$\text{mg cholesterol/dl} = T/S \times Cs \text{ (mg/dl)}$$

$$\text{mmol cholesterol/L} = \text{mg/dl} \times 0.026$$

Serum triglycerides (triacylglycerol) (TG): (bioMerieux kits)[123]

It was determined enzymatically without deproteinization according to the following reaction:

a- Enzymatic hydrolysis with lipase: $\text{TG} \xrightarrow{\text{lipase}} \text{glycerol} +$ fatty acids

b- Glycerol + ATP $\xrightarrow{\text{glycerol kinase}}$ glycerol-3-PO_4 + ADP

c- Glycerol-3-PO_4 $\xrightarrow{\text{glycerol-3-}PO_4\text{ oxidase}}$ dihydroxyacetone PO_4 + H_2O_2

d- Colour development:

$2H_2O_2$ + paracholorophenol + 4 amino antipyrene $\xrightarrow{\text{peroxidase}}$ quinoneinine (red coloured) + $4H_2O$

The rose coloured chromogen which is proportionate to TG concentration in the sample (T) was measured spectrophotometrically at λ 505nm and compared to a standard of known TG concentration (mg/dl) similarly treated (S).

TG level was obtained by the following equation:

$$mgTG/dl = T/S \times Cs\ (mg/dl)$$

$$mmol\ triglycerides/L = mg/dl \times 0.01145$$

High density lipoprotein (HDL) - cholesterol:[124]

It was determined by mixing the plasma with HDL precipitating mixture (phosphotungistic acid and magnesium chloride) that precipitates both VLDL and LDL together with chylomicrons in the sample.

In the supernatant obtained after centrifugation (containing the HDL), the cholesterol content was determined according to the Liebermann-Burchard (L-B) reaction and read spectrophotometrically at λ 620 nm (T), and compared to a standard cholesterol of known concentration (mg/dl) (S).

HDL cholesterol was obtained according to the following equation:

$$\text{mg HDL chol/dl} = T/S \times Cs \text{ (mg/dl)}$$

$$\text{mmol HDL-cholesterol/L} = \text{mg/dl} \times 0.026$$

Low density lipoprotein (LDL)- cholesterol:[125]

It was determined by mixing the plasma with heparin calcium chloride mixture which upon centrifugation left the HDL in the supernatant to be decanted.

Determination of LDL in the precipitate (pellet) was done according to the Liebermann-Burchard reaction (T), read spectrophotometrically at λ 620 nm, and compared to a known standard cholesterol solution (mg/dl) (S).

LDL chol was calculated as follows:

$$\text{mg LDL - chol/dl} = T/S \times Cs \text{ (mg/dl)}$$

$$\text{mmol LDL - chol/L} = \text{mg/dl} \times 0.026$$

Thiobarbituric acid reactive substances (TBARs):[126,127]

The thiobarbituric acid reactive substances assay measured the amount of malondialdehyde (MDA) and malondialdehyde like substances formed by the lipid peroxidation process.

It involved heating the sample under acidic condition with thiobarbituric acid (TBA) which reacts with MDA to form a red MDA-TBA complex (T) which was measured spectrophotometrically at λ 532 nm and compared to a standard similarly treated (S).

Reagents:

a- Thiobarbituric acid:

0.7% in 10% Trichloro acetic acid (0.7 gm TBA was dissolved in 100 ml 10% trichloroacetic acid).

b- Standard used was malondialdehyde-bis-diethyl acetate (1,1,3,3 tetraethoxypropane, (4 molar - 97% pure-density of 0.91).

The stock solution was diluted 1:1000 with 50 mmol/L (0.05 Molar) sulphuric acid (H_2SO_4) and from this dilution 0.25 ml was taken and completed to 50 ml in a volumetric flask with 50 mmol/L H_2SO_4.

With each run 2 standard concentrations were done, the first was equivalent to 20 µmol/L and the second was equivalent to 10 µmol/L as follows:

0.3 ml was taken from the last dilution which was equivalent to 20 µmol/L.

0.15 ml and completed to 0.3 ml with dist H_2O which was equivalent to 10 µmol/L.

Specimens:

Two ml heparinized blood were put on 50 µL of 3.8% EDTA (that acted as a preservative for TBARs), centrifuged and plasma was separated.

Procedure:

To 300 µL of plasma, 4 ml of thiobarbituric acid were added, in a centrifuge tube and incubated for 30 minutes at 100°C.

The tubes were left to cool down, centrifuged for 15 minutes at 1500 g and the supernatant was read spectrophotometrically at λ 532nm (T) using distilled water as a blank.

A similarly treated standard of a 10 µmol/L concentration was done with each run.

Calculation:

$$\mu mol\ TBARs/L = T/S \times 10$$

Serum Ferritin: (ELAgen kits)[128]

Principle:

The ELAgen ferritin is based on the principle of a solid phase enzyme-linked immunosorbent assay.

The assay utilizes rabbit anti-ferritin bound to solid phase microwells and mouse monoclonal antiferritin in the antibody-enzyme (horseradish peroxidase) conjugate solution.

The test sample is allowed to react simultaneously with the antibodies, resulting in the ferritin molecules being sandwiched between the solid phase and enzyme-linked antibodies.

After a 45 minute incubation at room temperature, the wells are washed with water to remove unbound antibody complexes.

A solution of 3,3',5,5' - tetramethylbenzidine (TMB) is added and incubated for 15 minutes, resulting in the development of a blue colour.

The colour development is stopped by the addition of stop solution, and the resulting yellow colour is measured spectrophotometrically at 450 nm.

The concentration of ferritin is directly proportional to the colour intensity of the test sample.

Reagents:

- EI Agen ferritin microplate:

The bag contains a microplate of 12 strips X 8 wells.

Each well is coated with rabbit antiferritin antibodies.

- EI Agen HRP-anti-ferritin conjugate:

The vial contains 13 ml of mouse monoclonal anti-ferritin conjugated to horseradish peroxidase.

Preservatives:

Gentamicin sulphate 0.005%, thimerosal 0.02%, phenol 0.006%, Kathon CG 0.002%. Ready to use.

- EI Agen ferritin standards:

The vials contain ferritin in bovine serum with preservatives, the concentrations of the standards are the following: 0,15,80,250,500 and 1000 ng/ml.

Lyophilized preservatives:

Gentamicin sulphate, 0.005%, thimerosal 0.02%, phenol 0.006%, Kathon CG 0.002%.

- EI Agen TMB H_2O_2 HS:

The vial contains 13 ml of a stabilized mixture of TMB (3,3',5,5' - tetramethylbenzidine) and H_2O_2 (hydrogen peroxide) ready to use.

- ELAgen stop solution (H_2SO_4):

The vial contains 13 ml of 0.3 M H_2SO_4. Ready to use.

Procedure:

1- The desired number of coated wells were secured in the holder.

2- 20 µL of standards, samples, and controls were dispenced into appropriate wells.

3- 20 µL of HRP-anti-ferritin conj. were dispenced into each well.

4- The plate was thoroughly shaked for 10 seconds.

5- Then the plate was incubated at room temperature for 45 minutes.

6- Incubation mixture was removed by flicking the plate contents into a suitable waste container.

7- The microwells were rinsed and flicked 5 times with distilled water.

8- The inverted wells were striked sharply on absorbent paper to remove residual water droplets.

9- 100 µL TMB substrate reagent were dispensed into each well.

10- The plate was incubated at room temperature in the dark for 15 minutes.

11- The reaction was stopped by adding 100 µL of stop solution to each well.

12- The absorbance was read at 450 nm.

Calculation:

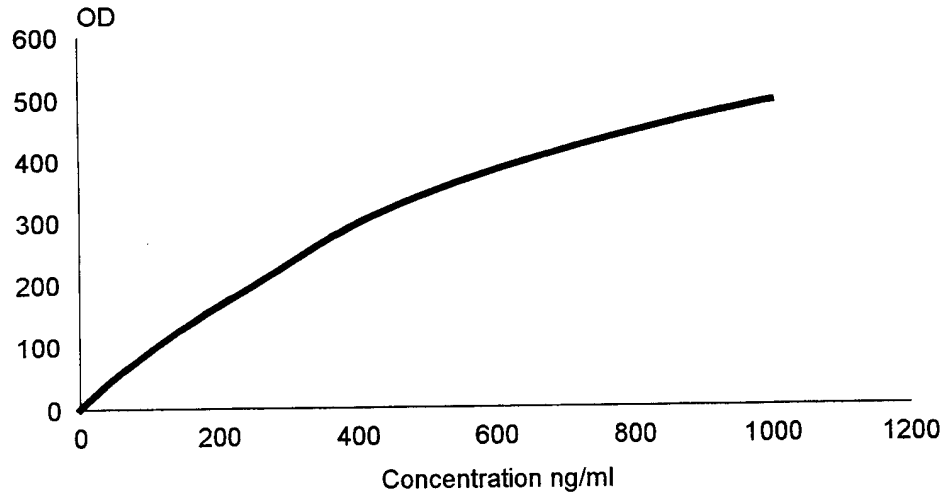
1- The mean absorbance values for the duplicate reference standards, controls and patient samples were calculated.

2- A standard curve was constructed on graph paper by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml. with OD on the vertical axis (y) and concentration on the horizontal axis (X).

3- The mean OD value for each sample was used to determine the corresponding concentration of ferritin in ng/ml from the standard curve.

4- Any diluted samples was multiplied by the appropriate dilution factor.

Ferritin standard curve.



Serum iron: (Ferrimat-kit)[129]

Principle:

In an acid medium and in the presence of guanidine, ferric ions are released from their protein bounds.

Using hydroxylamine, the iron is reduced from the ferric to the ferrous state which forms a coloured complex with ferrozine. The colour was read spectrophotometrically at λ 562 nm.

Calculation:

$$\mu g/dl = \frac{\text{A sample - A sample blank}}{\text{A standard}} \text{ X n}$$

n = 200

μmol iron /L = μg/dL X 0.179

Serum TIBC:[129]

Principle:

Serum total iron binding capacity was measured after saturation of the transferrin with iron solution.

The excess iron was adsorbed on magnesium hydroxycarbonate, then determination of iron was performed.

Calculation:

$$\mu g/dl = \frac{\text{A sample - A sample blank}}{\text{A standard}} \text{ X n}$$

n = 600

μmol TIBC/L = μg/dL X 0.179

$$\text{Transferrin \% saturation} = \frac{\text{serum iron concentration}}{\text{TIBC}} \text{ X } 100$$

Superoxide dismutase (SOD):[130]

Principle:

The method depend on the spontaneous autoxidation of pyrogallol at alkaline pH, resulting in the production of superoxide anion radical O_2^- which turn enhances the autoxidation of pyrogallol. Autoxidation is manifested as an increase in absorbance at 420 nm.

The presence of superoxide dismutase in the medium leads to removal of superoxide anion.

$$2\,\dot{O}_2^- + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

Thereby inhibiting the autoxidation of pyrogallol.

Reagents:

1- 50 nM tris Hcl buffer containing 1 mM DTPA (diethylene-triamino penta acetic acid).

2- 20 mM pyrogallol (1,2,3' benzentriol) in 10 mM Hcl. Stock solutions in 10 mM Hcl are stable for weeks.

3- Bovine Cu-Zn superoxide dismutase (erythrocuprein) was prepared from erythrocytes. A standard solution was prepared in double distilled H_2O.

Procedure:

Blank: in a spectrophotometer cuvette maintained at 30°C, 1ml of the tris Hcl buffer was added.

+ 10 µL pyrogallol.

The absorbance was followed for 1 min at 420 nm.

The rate of increase in absorbance under these condition was 0.02/min.

Standard:

The same procedure for the blank was repeated except the addition of SOD before recording absorbance.

- 1 ml tris buffer.
- 10 µL pyrogallol.
- 30 µL standard solution.

Sample: The procedure processed as standard.

SOD unit:

1 unit SOD activity is the amount of enzyme which inhibit the rate of autoxidation of pyrogallol by 50% from standard curve.

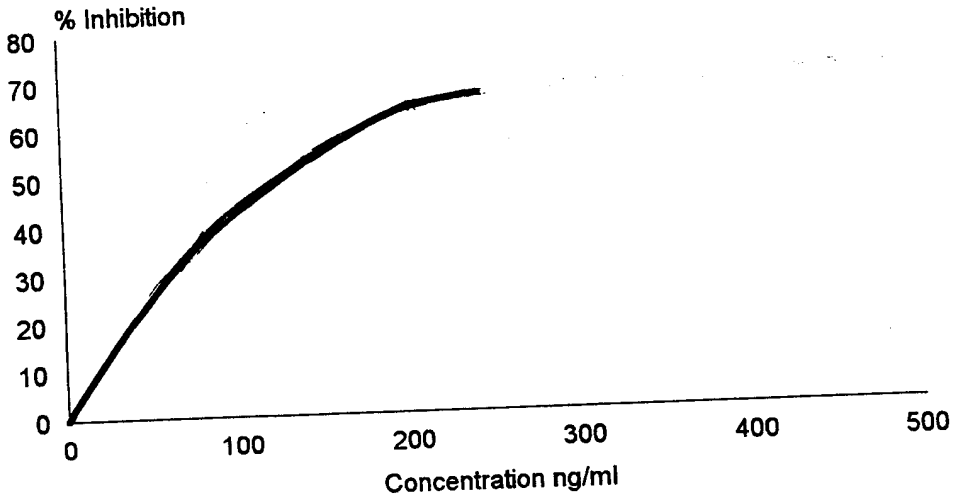
Calculation:

% of inhibition of pyrogallol autoxidation =

$$100 - \frac{\text{absorbance/min (standard or sample)}}{\text{absorbance/min (blank)}} \text{ X } 100$$

Standard curve was made by blotting the amount of SOD in (ng) versus % of inhibition of autoxidation. The amount of SOD was determined from standard curve and expressed in (ng/ml).

Standard curve of SOD.



Statistical analysis[131]

The row data were coded and transmitted into coding sheets. The coded data were typed onto a computer file using the D base program. The coded data were checked for transmission error. The data were analyzed using the SPSS statistical software. The personal computer IPM compatible was employed for statistical analysis, which include:

1- Arithmetic mean (X): It was calculated as follows:

$$X = \frac{\Sigma X}{n}$$

Where ΣX = sum of observations

n = number of observations.

2- Standard deviation (S.D.): It was calculated as follows:

$$S.D = \sqrt{\frac{\Sigma X^2 - (\Sigma X)^2 / n}{n-1}}$$

Where ΣX^2 = sum of squared observations

$(\Sigma X)^2$ = square of the sum of observations.

3- Standard error (S.E):

$$S.E. = \frac{S.D}{\sqrt{n}}$$

The results were considered significant if the probability "P" value was less than 0.05. The following tests of significance were used to study the association between different variables in the different groups:

4- Student "t" test:

It is a test of significance for the difference between two sample means.

It can be calculated from the following equation:

$$t = \frac{\bar{X_1} - \bar{X_2}}{\sqrt{(S.E_1)^2 + (S.E_2)^2}}$$

Where X_1= mean of the first sample

X_2= mean of the second sample

$S.E_1$= the standard error of the first sample

$S.E_2$= the standard error of the second sample

5- Pearson's correlation:

This measure reports the strength of the relationship between two variables.

The "r" can have any value from –1.00 to +1.00. It is obtained by:

$$r = \frac{n(\sum XY) - (\sum X)(\sum Y)}{\sqrt{(n(\sum X^2) - (\sum X)^2)(n(\sum Y^2) - (\sum Y)^2)}}$$

Where n = the number of paired observations

ΣX = the sum of the X-variable

ΣY = the sum of the Y variable

ΣX^2= the sum of the squared X-variable

$(\Sigma X)^2$= the square of the summed X-variable

ΣY^2= the sum of the squared Y-variable

$(\Sigma X)^2$= the square of the summed Y-variable.

RESULTS

RESULTS

Age and blood picture in the studied groups: (Table Ia,b)

Age:

In the present study control group consists of 10 subjects, their mean age value was 54±6.8 years, in the group of total patients with angina (30 subjects) their mean age value was 51.6±5 years, in the group of patients with stable angina the mean age value was 51.4±5.6 years and in the group of patients with unstable angina the mean age value was 51.7±4.6 years.

No significant difference between the studied groups was found.

Haemoglobin:

In the present study, control group showed a mean haemoglobin value of 13.7±0.88 G/dl while the group of whole patients with angina showed mean Hb value of 12.6±1.2 G/dl. The group of patients with stable angina showed mean Hb value of 12.5±1.1 G/dl. and in the group of patients with unstable angina the mean Hb value was 12.5±1.2 G/dl.

A significant difference was found when the total patients with angina group, patients with stable angina group and patients with unstable angina group were compred with the control group ($P<0.01$).

No significant difference was found between the group of patients with stable angina and the group of patients with unstable angina.

Haematocrit:

In the present study control group showed mean Ht value of 41±2.3%.

In group of total patients with angina mean Ht value was 37.4±4.6%. This was significantly lower than that of the control group ($P<0.01$).

In group of patients with stable angina mean Ht value was 36.9±4.9%. This was significantly lower than that of the control group ($P<0.01$).

In group of patient with unstable angina mean Ht value was 37.9±4.5%. This was significantly lower than that of the control group ($P<0.05$).

No significant difference was found between the group of patients with stable angina when compared with the group of patients with unstable angina.

Table I a: Age, Blood haemoglobin (Hb) g/dl and Haematocrit (Ht) % in controls (group I) and total patients with angina (group II).

Case No.	Controls			Total		
	Age	Hb G/dl	Ht %	Age	Hb G/dl	Ht %
1	55	13.3	40	59	14.5	44
2	45	12.8	39	56	13.8	41
3	50	15	45	43	13	40
4	60	13	40	56	13.5	40
5	65	12.9	39	59	14	42
6	57	12.8	38	46	13	40
7	58	14.4	41	49	11.1	32
8	60	14	41	55	12	32
9	46	14.2	43	59	11.1	30
10	47	15	44	46	11.7	32
11				50	12.8	39
12				49	12.2	39
13				47	13.3	40
14				53	11.1	30
15				45	11.8	32
16				54	11.1	31
17				55	12	39
18				56	14.5	43
19				57	13	40
20				45	12	39
21				46	13.2	40
22				53	12.8	40
23				51	12.8	39
24				52	11	30
25				47	11	31
26				54	14	41
27				55	11	32
28				47	13	40
29				59	14.6	44
30				45	12.2	39
X	54	13.7	41	51.6	12.6	37.4
SD	6.8	0.88	2.3	5	1.2	4.6
P1				Ns	<0.01	<0.01

P1: Statistical significance between the control group and the group of total patients with angina.

Table Ib: Age, Blood haemoglobin (Hb) g/dl and Haemãtocrit (Ht) % in patients with stable angina (group III) and patients with unstable angina (group IV).

Case No.	Patients with stable angina			Patients with unstable angina		
	Age	Hb G/dl	Ht %	Age	Hb G/dl	Ht %
1	59	14.5	44	54	11.1	31
2	56	13.8	41	55	12	39
3	43	13	40	56	14.5	43
4	56	13.5	40	57	13	40
5	59	14	42	45	12	39
6	46	13	40	46	13.2	40
7	49	11.1	32	53	12.8	40
8	55	12	32	51	12.8	39
9	59	11.1	30	52	11	30
10	46	11.7	32	47	11	31
11	50	12.8	39	54	14	41
12	49	12.2	39	55	11	32
13	47	13.3	40	47	13	40
14	53	11.1	30	59	14.6	44
15	45	11.8	32	45	12.2	39
X	51.4	12.5	36.9	51.7	12.5	37.9
SD	5.6	1.1	4.9	4.6	1.2	4.5
P2	Ns	<0.01	<0.01			
P3				Ns	<0.01	<0.05
P4				Ns	Ns	Ns

P2: Statistical significance between the control group and the group of patients with stable angina.
P3: Statistical significance between the control group and the group of patients with unstable angina.
P4: Statistical significance between the group of patients with stable angina and the group of patients with unstable angina.

Fasting blood glucose, urea, creatinine levels (mmol/L) in the studied groups (Table IIa,b):

Fasting blood glucose:

In the present study the control group showed mean value of FBG 4.7±0.53 mmol/L. The group of total patients with angina showed mean value of 4.8±0.58 mmol/L, the group of patients with stable angina showed mean value of 4.95±0.6 mmol/L and in the group of patients with unstable angina the mean value of FBG was 4.7±0.55 mmol/L.

No significant difference was found between the studied groups.

Urea:

In the present study the mean value of urea was 4.8±0.46 mmol/L in the control group, was 5.14±0.96 mmol/L in the group of total patients with angina, was 5.4±0.86 mmol/L in the group of patient with stable angina, and 4.9±1 mmol/L in the group of patients with unstable angina.

No significant difference was found between the studied groups.

Creatinine:

In the present study the control group showed mean value of creatinine of 0.079±0.0166 mmol/L, the group of total patients with angina showed mean value of 0.072±0.0179 mmol/L, the group of patients with stable angina showed mean value of 0.076±0.0164

mmol/L and the mean value of creatinine in the group of patients with unstable angina was 0.068±0.019 mmol/L

No significant difference was found between the studied groups.

Table II a: Fasting blood glucose, urea and creatinine levels (mmol/L) in controls (group I) and total patients with angina (group II).

Case No.	Controls			Total		
	FBG mmol/L	Urea mmol/L	Cr mmol/L	FBG mmol/L	Urea mmol/L	Cr mmol/L
1	4.24	5.16	0.10	4.18	6.49	0.10
2	4.84	4.83	0.08	5.50	5.66	0.08
3	5.28	5.66	0.09	4.13	4.16	0.07
4	5.34	4.50	0.06	4.45	5.33	0.06
5	5.34	5.33	0.05	4.73	5.99	0.07
6	4.46	5.00	0.10	6.05	4.50	0.06
7	3.85	4.33	0.09	5.28	5.99	0.07
8	4.57	4.83	0.07	5.45	6.49	0.10
9	4.18	4.66	0.07	4.56	4.50	0.10
10	4.40	4.16	0.08	4.70	4.83	0.08
11				5.56	5.66	0.08
12				4.68	5.00	0.07
13				4.84	6.66	0.08
14				4.40	5.49	0.08
15				5.78	4.00	0.04
16				4.95	6.16	0.08
17				4.51	4.50	0.08
18				4.35	4.66	0.08
19				4.07	6.16	0.05
20				4.79	5.99	0.08
21				4.07	5.33	0.10
22				5.61	5.83	0.04
23				5.39	3.50	0.04
24				4.24	5.33	0.08
25				4.46	3.33	0.05
26				4.68	5.66	0.04
27				5.34	3.83	0.07
28				4.68	3.50	0.08
29				3.96	5.33	0.08
30				5.50	4.33	0.07
X	4.70	4.80	0.079	4.80	5.14	0.072
SD	0.53	0.46	0.0166	0.58	0.96	0.0179
P1				Ns	Ns	Ns

P1: Statistical significance between the control group and the group of total patients with angina.

Table II b: Fasting blood glucose, urea and creatinine levels (mmol/L) in patients with stable angina (group III) and patients with unstable angina (group IV).

Case No.	Patients with stable angina			Patients with unstable angina		
	FBG mmol/L	Urea mmol/L	Cr mmol/L	FBG mmol/L	Urea mmol/L	Cr mmol/L
1	4.18	6.49	0.10	4.95	6.16	0.08
2	5.50	5.66	0.08	4.51	4.50	0.08
3	4.13	4.16	0.07	4.35	4.66	0.08
4	4.45	5.33	0.06	4.07	6.16	0.05
5	4.73	5.99	0.07	4.79	5.99	0.08
6	6.05	4.50	0.06	4.07	5.33	0.10
7	5.28	5.99	0.07	5.61	5.83	0.04
8	5.45	6.49	0.10	5.39	3.50	0.04
9	4.56	4.50	0.10	4.24	5.33	0.08
10	4.70	4.83	0.08	4.46	3.33	0.05
11	5.56	5.66	0.08	4.68	5.66	0.04
12	4.68	5.00	0.07	5.34	3.83	0.07
13	4.84	6.66	0.08	4.68	3.50	0.08
14	4.40	5.49	0.08	3.96	5.33	0.08
15	5.78	4.00	0.04	5.50	4.33	0.07
X	4.95	5.4	0.076	4.7	4.9	0.068
SD	0.6	0.86	0.0164	0.55	1.0	0.019
P2	Ns	Ns	Ns			
P3				Ns	Ns	Ns
P4				Ns	Ns	Ns

P2: Statistical significance between the control group and the group of patients with stable angina.
P3: Statistical significance between the control group and the group of patients with unstable angina.
P4: Statistical significance between the group of patients with stable angina and the group of patients with unstable angina.

Blood lipid (cholesterol – HDL-C, LDL-C, TG) levels mmol/L in the studied groups Table III (a,b):

Total cholesterol:

Mean total cholesterol value was 4.3±0.6 mmol/L in the control group, 6.3±1.5 mmol/L, 6.3±1.7 mmol/L and 6.3±1.3 mmol/L in the group of total patients with angina, stable angina group and unstable angina group respectively.

Significantly higher values was found in these three groups when compared with control group ($P<0.001$).

High density lipoprotein cholesterol:

Mean HDLC value was 1.3±0.2 mmol/L in the control group, 1±0.2 mmol/L and 1±0.2 mmol/L (in group of total patients with angina and group of patients with stable angina). Significantly lower values was found between these two groups when compared with the control group ($P<0.01$), and 1.03±0.27 mmol/L in group of patients with unstable angina and this was significantly lower than that of the control group ($P<0.02$).

Low density lipoprotein cholesterol:

Mean LDLC value was 2.4±0.5 mmol/L in the control group, 4.7±1.3 mmol/L, 4.66±1.5 mmol/L and 4.7±1.1 mmol/L in (group of total patients with angina, stable angina group and unstable angina group) respectively.

Significantly higher values was found in these three groups when compared with the control group ($P<0.001$).

Triglycerides:

Mean TG value was 1.1±0.3 mmol/L in the control group, 1.4±0.6 mmol/L in the group of total patients with angina and this was significantly higher than that of control group ($P<0.05$).

Mean TG level was 1.47±0.6 mmol/L in the group of patients with stable angina and 1.4±0.7 mmol/L in the group of patients with unstable angina.

No significant difference was found in these two groups when compared with the control group.

Table III a: Blood lipid levels (mmol/L) in controls (group I), and total patients with angina (group II).

Case No.	Controls				Total			
	TCh mmol/L	HDL-C mmol/L	LDL-C mmol/L	TG Mmol/L	TCh mmol/L	HDL-C mmol/L	LDL-C mmol/L	TG mmol/L
1	4.18	1.03	2.63	1.20	6.81	1.03	5.29	1.14
2	4.08	1.50	2.12	1.02	5.16	1.19	3.56	0.96
3	3.48	1.03	2.06	0.90	4.88	1.03	3.20	1.44
4	3.95	1.37	1.86	1.56	8.67	1.03	6.97	1.50
5	4.52	1.47	2.45	1.32	6.35	0.98	4.80	1.30
6	4.08	1.06	2.50	1.19	3.87	0.95	2.45	1.08
7	3.64	1.60	1.73	0.72	4.90	0.90	3.59	0.96
8	4.21	1.03	2.58	1.32	9.29	1.08	7.48	1.68
9	5.31	1.34	3.35	1.44	7.22	1.06	5.55	1.44
10	5.21	1.86	3.04	0.72	3.56	0.67	2.37	1.18
11					5.42	1.01	4.00	0.96
12					7.74	1.08	5.83	1.92
13					6.19	1.55	3.72	2.16
14					6.66	1.03	5.06	1.30
15					8.05	0.65	6.06	3.12
16					7.22	1.44	5.16	1.44
17					5.16	0.70	4.00	1.08
18					5.16	1.19	3.56	0.96
19					5.93	1.03	4.23	1.56
20					6.97	0.93	5.42	1.44
21					7.61	0.85	5.83	2.16
22					5.73	1.60	3.61	1.20
23					5.42	0.70	4.41	0.72
24					5.16	1.01	3.74	0.84
25					4.64	0.77	3.61	0.60
26					6.71	1.39	4.90	0.96
27					7.74	0.83	5.47	3.36
28					7.53	0.93	5.68	2.16
29					5.06	1.03	3.61	0.96
30					9.29	1.08	7.48	1.68
X	4.3	1.3	2.4	1.1	6.3	1.0	4.7	1.4
SD	0.6	0.2	0.5	0.3	1.5	0.2	1.3	0.6
P1					<0.001	<0.01	<0.001	<0.05

P1: Statistical significance between the control group and the group of total patients with angina.

Table III b: Blood lipid levels (mmol/L) in patients with stable angina (group III) and patients with unstable angina (group IV).

Case No.	Patients with stable angina				Patients with unstable angina			
	TCh mmol/L	HDL-C mmol/L	LDL-C mmol/L	TG mmol/L	TCh mmol/L	HDL-C mmol/L	LDL-C mmol/L	TG mmol/L
1	6.81	1.03	5.29	1.14	7.22	1.44	5.16	1.44
2	5.16	1.19	3.56	0.96	5.16	0.70	4.00	1.08
3	4.88	1.03	3.20	1.44	5.16	1.19	3.56	0.96
4	8.67	1.03	6.97	1.50	5.93	1.03	4.23	1.56
5	6.35	0.98	4.80	1.30	6.97	0.93	5.42	1.44
6	3.87	0.95	2.45	1.08	7.61	0.85	5.83	2.16
7	4.90	0.90	3.59	0.96	5.73	1.60	3.61	1.20
8	9.29	1.08	7.48	1.68	5.42	0.70	4.41	0.72
9	7.22	1.06	5.55	1.44	5.16	1.01	3.79	0.84
10	3.56	0.67	2.37	1.18	4.64	0.77	3.61	0.60
11	5.42	1.01	4.00	0.96	6.71	1.39	4.90	0.96
12	7.74	1.08	5.83	1.92	7.74	0.83	5.47	3.36
13	6.19	1.55	3.72	2.16	7.53	0.93	5.68	2.16
14	6.66	1.03	5.06	1.30	5.06	1.03	3.61	0.96
15	8.05	0.65	6.06	3.12	9.29	1.08	7.48	1.68
X	6.3	1.0	4.66	1.47	6.3	1.03	4.7	1.4
SD	1.7	0.2	1.5	0.6	1.3	0.27	1.1	0.7
P2	<0.001	<0.01	<0.001	Ns				
P3					<0.001	<0.02	<0.001	Ns
P4					Ns	Ns	Ns	Ns

P2: Statistical significance between the control group and the group of patients with stable angina.
P3: Statistical significance between the control group and the group of patients with unstable angina.
P4: Statistical significance between the group of patients with stable angina and the group of patients with unstable angina.

Iron status in the studied groups (Table IV a,b):

Serum iron:

In the present study, control group showed a mean serum iron value of 16.3±2.2 µmol/L, total patients with angina group showed a mean value of 17.35±4.8 µmol/L, patients with stable angina group showed a mean value of 17.7±4.6 µmol/L and patients with unstable angina showed a mean value of 16.9±5.1 µmol/L.

No significant difference was found was found between the studied groups.

TIBC:

In the present study, control group showed a mean TIBC value of 56.9±12.2 µmol/L, group of total patients with angina showed a mean value of 63.1±7.6 µmol/L, patients with stable angina group showed a mean value of 62.1±8.2 µmol/L and the group of patients with unstable angina showed a mean value of 64±7 µmol/L.

No significant difference was found between the studied groups.

Ferritin:

In the present study, control group showed a mean ferritin level of 62.5±13.8 µg/L. Mean ferritin levels were 138.1±60 µg/L, 142.9±69.75 µg/L and 133±50.9 µg/L in the total angina group patients, stable angina group and unstable angina group respectively. The levels were significantly higher in these 3 groups when compared to the control group ($p<0.001$).

Table IV a: Iron status in controls (group I) and total patients with angina (group II).

Case No.	Controls			Total		
	Fe µmol/L	TIBC µmol/L	Ferritin µg/L	Fe µmol/L	TIBC µmol/L	Ferritin µg/L
1	21.48	72.50	59	25.42	72.32	270
2	14.32	62.65	60	24.34	71.06	210
3	15.93	75.18	50	19.51	67.48	105
4	16.83	63.00	37	22.38	68.92	97
5	15.93	40.63	80	22.38	68.92	199
6	17.90	62.47	55	17.90	63.36	99
7	15.75	44.03	59	10.74	47.44	85
8	14.68	42.07	70	12.89	49.23	224
9	16.29	52.98	80	10.74	47.44	100
10	13.43	54.24	75	15.75	61.22	90
11				16.83	62.47	110
12				15.75	60.86	100
13				19.51	67.66	85
14				17.72	62.83	100
15				13.78	59.97	270
16				11.28	48.87	90
17				12.53	59.97	130
18				25.06	71.60	115
19				20.94	64.44	225
20				12.89	49.23	250
21				20.94	64.44	95
22				16.29	63.55	95
23				16.11	63.55	105
24				11.64	66.23	110
25				11.64	65.34	145
26				23.27	70.71	190
27				11.99	70.88	100
28				18.80	64.44	120
29				25.06	72.50	80
30				16.29	64.44	150
X	16.3	56.9	62.5	17.35	63.1	138.1
SD	2.2	12.2	13.8	4.8	7.6	60
P1				Ns	Ns	<0.001

P1: Statistical significance between the control group and the group of total patients with angina.

Table IV b: Iron status in patients with stable angina (group III) and patients with unstable angina (group IV).

Case No.	Patients with stable angina			Patients with unstable angina		
	Fe µmol/L	TIBC µmol/L	Ferritin µg/L	Fe µmol/L	TIBC µmol/L	Ferritin µg/L
1	25.42	72.32	270	11.28	48.87	90
2	24.34	71.06	210	12.53	59.97	130
3	19.51	67.48	105	25.06	71.60	115
4	22.38	68.92	97	20.94	64.44	225
5	22.38	68.92	199	12.89	49.23	250
6	17.90	63.36	99	20.94	64.44	95
7	10.74	47.44	85	16.29	63.55	95
8	12.89	49.23	224	16.11	63.55	105
9	10.74	47.44	100	11.64	66.23	110
10	15.75	61.22	90	11.64	65.34	145
11	16.83	62.47	110	23.27	70.71	190
12	15.75	60.86	100	11.99	70.88	100
13	19.51	67.66	85	18.80	64.44	120
14	17.72	62.83	100	25.06	72.50	80
15	13.78	59.97	270	16.29	64.44	150
X	17.7	62.1	142.9	16.9	64	133
SD	4.6	8.2	69.75	5.1	7	50.9
P2	Ns	Ns	<0.001			
P3				Ns	Ns	<0.001
P4				Ns	Ns	Ns

P2: Statistical significance between the control group and the group of patients with stable angina.
P3: Statistical significance between the control group and the group of patients with unstable angina.
P4: Statistical significance between the group of patients with stable angina and the group of patients with unstable angina.

Plasma thiobarbituric acid reactive substances levels (μmol/L) in the studied groups (Table Va,b):

Mean plasma TBARs value was 2.3±0.78 μmol/L in control group, 3.6±1.4μmol/L in the group of total patients with angina and it was significantly higher than that of the control group ($p<0.01$). It was 3.4±1.5 μmol/L in stable angina group, and it was significantly higher than that of control group ($p<0.05$) and was 3.7±1.2 μmol/L in unstable angina group and it was significantly higher than that of control group ($P<0.01$).

Table V a: Plasma thiobarbituric acid reactive substances (TBARs) levels (μmol/L) in controls (group I) and total patients with angina (group II).

Case No.	Controls	Total
	TBARs μmol/L	TBARs μmol/L
1	1.2	3.4
2	1.8	5.4
3	2.4	2.0
4	3.5	3.5
5	2.5	2.2
6	2.7	3.3
7	1.4	3.8
8	3.0	5.6
9	3.3	2.5
10	1.9	6.2
11		2.0
12		2.0
13		5.4
14		2.2
15		2.3
16		3.3
17		3.9
18		6.0
19		4.1
20		2.3
21		2.9
22		2.1
23		2.5
24		5.3
25		3.8
26		3.9
27		3.0
28		3.4
29		6.2
30		3.5
X	2.3	3.6
SD	0.78	1.4
P1		<0.01

P1: Statistical significance between the control group and the group of total patients with angina.

Table V b: Plasma thiobarbituric acid reactive substances (TBARs) levels (μmol/L) in patients with stable angina (group III) and patients with unstable angina (group IV).

Case No.	Patients with stable angina	Patients with unstable angina
	TBARs μmol/L	TBARs μmol/L
1	3.4	3.3
2	5.4	3.9
3	2.0	6.0
4	3.5	4.1
5	2.2	2.3
6	3.3	2.9
7	3.8	2.1
8	5.6	2.5
9	2.5	5.3
10	6.2	3.8
11	2.0	3.9
12	2.0	3.0
13	5.4	3.4
14	2.2	6.2
15	2.3	3.5
X	3.4	3.7
SD	1.5	1.2
P2	<0.05	
P3		<0.01
P4		Ns

P2: Statistical significance between the control group and the group of patients with stable angina.
P3: Statistical significance between the control group and the group of patients with unstable angina.
P4: Statistical significance between the group of patients with stable angina and the group of patients with unstable angina.

Serum superoxide dismutase (SOD) level (ng/ml) in the studied groups (Table VI a,b):

In the present study mean serum SOD value was 30±8.6 ng/ml in the control group, 61.8±13.3 ng/ml, 60.8±14.6 ng/ml and 62.7±12.4 ng/ml in the group of total patients with angina, stable angina group, unstable angina group respectively.

Significantly higher values where found in these three groups when compared with the control group ($P<0.001$).

Table VI a: Serum superoxide dismutase (SOD) levels (ng/ml) in controls (group I) and total patients with angina (group II).

Case No.	Controls	Total
	SOD ng/ml	SOD ng/ml
1	35	73
2	30	60
3	26	56
4	40	73
5	17	75
6	21	40
7	40	45
8	30	90
9	21	71
10	40	65
11		45
12		50
13		40
14		65
15		65
16		62
17		58
18		73
19		59
20		58
21		45
22		73
23		56
24		71
25		56
26		40
27		65
28		60
29		90
30		75
X	30	61.8
SD	8.6	13.3
P1		<0.001

P1: Statistical significance between the control group and the group of total patients with angina.

Table VI b: Serum superoxide dismutase (SOD) levels (ng/ml) in patients with stable angina (group III) and patients with unstable angina (group IV).

Case No.	Patients with stable angina	Patients with unstable angina
	SOD ng/ml	SOD ng/ml
1	73	62
2	60	58
3	56	73
4	73	59
5	75	58
6	40	45
7	45	73
8	90	56
9	71	71
10	65	56
11	45	40
12	50	65
13	40	60
14	65	90
15	65	75
X	60.8	62.7
SD	14.6	12.4
P2	<0.001	
P3		<0.001
P4		Ns

P2: Statistical significance between the control group and the group of patients with stable angina.
P3: Statistical significance between the control group and the group of patients with unstable angina.
P4: Statistical significance between the group of patients with stable angina and the group of patients with unstable angina.

DISCUSSION

DISCUSSION

Extensive reviews have been published concerning the role of iron in free radical reactions, such as lipid peroxidation. Briefly, except in states of iron overload, all iron in human serum is bound to proteins. (39, 132-134)

About two thirds of body iron is found in hemoglobin, with smaller amounts in myoglobin, various enzymes, and the transport protein transferrin. Iron not required for these is largely stored in ferritin.[39]

To promote free radical production, iron must be liberated from proteins. It is believed that oxidant stress itself can provide the iron necessary for formation of reactive oxygen species, for example, by mobilizing iron from ferritin or by degrading heme proteins to release iron.[39] For instance, superoxide radicals have been observed to liberate iron from ferritin, promoting lipid peroxidation.[39,135]

Halliwell and Gutteridge have proposed that the general effect of iron catalysts is to convert poorly reactive free radicals into highly reactive ones, such as the hydroxyl radical.[39]

Balla and Coworkers[136] demonstrated that the combination of physiological concentrations of hydrogen peroxide and hemin induce a rapid peroxidation of LDL in vitro and that free iron is released from the degraded heme ring. They also observed a simultaneous loss of reactive lysine amino groups of LDL, which has

simultaneous loss of reactive lysine amino groups of LDL, which has been shown to promote LDL uptake by scavenger receptors of macrophages leading to foam cell formation.[137]

Also transition metal ions are probably required for the peroxidation of LDL by monocyte/macrophages, smooth muscle cells, and endothelial cells.[138,139]

There is little doubt that an excessive amount of stored iron is cardiotoxic. Myocardial failure is a prominent feature of haemochromatosis and in diseases associated with large exogenous iron loads, such as thalassaemia major.[140-142]

Patients with these diseases usually have iron stores many times higher than normal. The mechanism of myocardial damage by iron is not clearly understood. It is dependent on poorly defined effects within the myocardium, where iron either damages the muscle itself or excites fibrosis.[143]

A role for iron in the development of coronary lesions is not indispensable to the hypothesis that iron depletion protects against IHD. Iron depletion could exert a protective effect by mechanisms that don't involve effects on atherogenesis for example, by decreasing the vulnerability of the myocardium to ischemia.[144]

Iron depletion could inhibit atherogenesis through a variety of mechanisms including, for example, protection of endothelial cells from

oxygen radical injury[145] or inhibition of low density lipoprotein modification.[146,147]

Salonen et al,[148] reported that dietary iron intake to be positively associated with an increased risk of myocardial infarction. In contrast, Rimm et al,[149] Cooper and Liao[150] reported no association between dietary iron intake and the risk of coronary heart disease.

In the present study, when comparing the serum iron level in CHD patients with the corresponding values of control group, it was noticed that the serum iron level was (17.7±4.6 mmol/L in stable group - 16.9±5.1 mmol/L in unstable group - 17.35±4.8 mmol/L in total group) and 16.3±2.2 mmol/L in control group and the difference was not statistically significant.

Also the values of serum TIBC in CHD patients (62.1±8.2 mmol/L, 64±7 mmol/L, 63.1±7.6 mmol/L in stable, unstable and total groups respectively) was mildly higher than that of the control group (56.9±12.2 mmol/L), but not to a significant degree.

These results are in agreement with study of Christopher et al,[151] several studies, which suggested that neither serum iron nor TIBC are related to the risk of CHD.

Normal serum iron, and TIBC levels noticed in the patient group of this study with elevated serum ferritin level could be due to the fact

that the effective iron in the process of lipid peroxidation is either the free iron or the ferritin molecule itself.

This can be further evidenced by the absence of any correlation between both serum iron, TIBC on one hand and the rest of risk factors (TCh, LDL-C, TG) on the other hand.

The concentration of serum ferritin in healthy individuals is positively correlated with the body iron stores.[152] In acute inflammation the serum ferritin level increases concomitantly with a decrease in serum iron acting as an acute phase reactant.[153]

Finlayson and Fraser had measured the ferritin level during the 1st 48 hours post AMI and found that the serum ferritin level gradually increases following AMI.[154]

The lack of acidic type ferritin in the serum (derived from myocardial cells) of AMI patients as tested by specific anti-heart and antiplacental ferritin McABS argues against the possibility that ferritin is released from damaged myocardial cells following infarction. This assumption is supported by the study of Cavanna et al, who reported a lack of heart ferritin in sera of patients examined 48 hours Post AMI.[155]

It has been reported that inflammation causes accumulation of iron in the reticuloendothelial cells, which may serve as an intracellular stimulus for ferritin synthesis.[156]

Inflammatory processes are characterized by an increase of mononuclear cells in the damaged area. In this sense, the iron binding neutrophilic glycoprotein, lactoferrin, plays an important role in the initial interaction with monocytes/macrophages during inflammation and is responsible for the inflammatory-reduced hyposideremia and accumulation of iron in these cells.[157]

Theoretically, ferritin as an iron binding protein may be considered as one of the primary antioxidants,[158] but many evidences contrast this hypothesis.[148,159,160]

Ferritin may be considered as a dangerous protein, as under certain conditions, the active oxygen species (superoxides) enter the ferritin-cores through the hydrophilic channels, followed by reduction of Fe(III) to Fe(II). This enables the release of free iron with its damaging effects.[148]

Nonenzymatic reduction of Fe(III) to Fe(II) occurs as iron is released from ferritin, thus ferritin is both a very efficient iron trap and a readily available source of iron for metabolic requirements and for formation of hemoglobin and other haemproteins.[93]

Salonen et al.,[148] reported an increased risk of acute myocardial infarction among men with serum ferritin level greater than 200 μg/L, where as preliminary results reported by Stampfer et al. did not support an increase in this risk.[161]

In the present work serum ferritin level was significantly higher in the angina patient group either stable (142.9±69.75 μg/L) or unstable (133±50.9μg/L) or the whole group (138.1±60 μg/L) compared to the corresponding control value (62.5±13.8 μg/L). This goes in hand with the elevated atherosclerotic risk lipid factors (cholesterol, LDL-C) and the diminished antirisk factor (HDL-C) as will be shown after words. It was noticed that the lipid profile of controls shows normal distribution and normal levels of different components. Their iron and ferritin levels where within the expected normal range.

There is a positive correlation between ferritin level and each of total cholesterol r (0.756) and LDL-C r (0.732). This would indicate the protective role of ferritin acting as an iron trap which would prevent its role in lipid peroxidation, hence guard against their atherogenic effects.

The present study revealed the following:

- A significant increase in serum total cholesterol level in CHD patients (6.3±1.7 mmol/L in stable group - 6.3±1.3 mmol/L in unstable group - 6.3±1.5 mmol/L in total group) more than controls (4.23±0.6 mmol/L).

- A significant increase in serum LDL-C level in CHD patients either stable group (4.66±1.5 mmol/L) or unstable group (4.7±1.1 mmol/L) or

the whole group (4.7±1.3 mmol/L) more than controls (2.4±0.5 mmol/L).

In the control group TBARs mean level was (2.3±0.78 µmol/L) and in the CHD patients, the mean level was (3.4±1.5 µmol/L) in stable group, (3.7±1.2 µmol/L) in unstable group (3.6±1.4 µmol/L) in the whole group, which were significantly higher than that of the control group.

It has been proposed that alteration of LDL-C produces clearance by subendothelial macrophages and result in intracellular deposition of lipoprotein-derived cholesterol.[162]

A study in vitro had implicated modification of LDL-C by lipid peroxide products as one potential mechanism.[163] MDA is an end product of lipid peroxidation. Reaction of MDA with a critical number of lysine residues of the apolipoprotein B-100 of LDL-C produce internalization by the scavenger receptor of human monocyte-macrophages and the subsequent intracellular accumulation of lipoprotein-derived cholesterol ester in vitro.[164]

Other study revealed that higher serum MDA, LDL-C levels and correlation of serum MDA with the extent of CA lesions exist in patients with CHD during both the early and late stages of coronary atherosclerosis, suggesting the existence in vivo of protein modified by a physiological product of lipid peroxidation within arterial lesion.[165]

In the present study, HDL-C was significantly lower in CHD patients (1.0±0.2 mmol/L) in stable group, (1.03±0.27 mmol/L) in unstable group, (1.0±0.2 mmol/L) in total group than controls (1.3±0.2 mmol/L).

Serum TG level was higher in CHD patient either stable (1.47±0.6 mmol/L) or unstable (1.4±0.7 mmol/L) than controls (1.1±0.3 mmol/L) but not to a significant degree. In the whole group the level was (1.4±0.6 mmol/L) which is significantly higher than controls.

It has been shown that triglycerides are not an independent cardiovascular risk factor.[166] In addition, there is some limited evidence suggesting that the risk of CHD increases as triglyceride level increases in patients exhibiting high levels of total cholesterol or LDL-C and low levels of HDL-C.[167]

Superoxide dismutase (SOD) catalyzes the dismutation of the superoxide anion radical to hydrogen peroxide and oxygen.

$$2O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$ [168]

Removal of excess O_2 by SOD is an important antioxidant defense mechanism in aerobic organisms.[42,169] SOD is ubiquitous in oxygen-metabolizing cells, protecting against oxygen toxicity.[170-173]

Mn-SOD can be considered as an enzyme specific for mitochondria that could be used as a blood marker for cardiac mitochondrial damage as it is located predominantly in the mitochondria and is present in the heart in high concentrations.[174-178]

The rise in SOD observed in the present study in all patient groups denotes an active antioxidant mechanism that tries to counter act the risky lipid and ferritin adverse effects.

In the group of stable angina, they showed a significant correlation between SOD and TCh - LDL-C and ferritin indicating that SOD activity keeps pace with these risk factors, hence counteract their adverse effects.

Such correlations disappeared in the group of unstable angina patients, it could be postulated that failure of SOD protective action may play a role in the development of unstable condition.

Levels of SOD in CHD patient groups was (60.8±14.6 ng/ml) in stable group, (62.7±12.4 ng/ml) in unstable group, (61.8±13.3 ng/ml) in total group than controls (30±8.6 ng/ml).

SOD had been reported to rise in serum after AMI, remains high for 2-7 days then returns to basal level. The cause for this rise is thought to be due to its release from myocardial tissue (mitochondria), that takes longer time to pass to blood stream than CK-MB.[179]

This transient rise in SOD level following AMI, differs from the rise noticed in its level in the patient groups (stable, unstable or total) reported in the present work which is not produced as a result of cell injury (which actually does not exist in such patients) but rather to a process of over production, over activity to counteract the adverse oxidative stress evidenced by the higher cholesterol, LDL-C, TG, ferritin and TBARs.

Summary & Conclusion

SUMMARY AND CONCLUSION

The oxidative modification of low density lipoprotein, as well as, its uptake by macrophages in vessel wall via scavenger receptors are considered important steps in the development of the atheromatous lesion. This uptake is not down regulated by the internalized cholesterol and cholesteryl esters and lead to their transformation to cells with the characteristic properties of lipid laden foam cells.

It has been shown that low density lipoprotein modification is due to free radical reaction and both copper and iron in reduced forms are able to induce such process.

To promote free radical production, iron must be liberated from proteins. It is believed that oxidant stress itself can provide the iron necessary for formation of reactive oxygen species, for example, by mobilizing iron from ferritin or by degrading heme proteins to release iron.

Theoretically, ferritin as an iron binding protein may be considered as one of the primary antioxidants that inhibits lipid peroxidation but many evidences contrast this hypothesis.

A reductive mechanism is probably responsible for the release of iron from ferritin into the low molecular weight pool, where it could induce lipid peroxidation and subsequent tissue injury by Fenton type reaction.

The aim of the present work is to study serum iron and ferritin in serum of patients with ischemic heart disease in a trial to evaluate their possible role in coronary atherosclerosis.

The present study was conducted on thirty patients with ischemic heart disease admitted to cardiology unit in the medical research institute as well as ten apparently normal healthy control subjects.

The selected patients included 15 with unstable angina pectoris and 15 with stable angina.

To all studied subjects included in the present study the following was done: full clinical examination, electrocardiogram, laboratory investigation including complete blood picture, complete urine analysis, serum fasting glucose, urea, creatinine, total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, triglycerides, iron, ferritin, superoxide dismutase and plasma thiobarbituric acid reactive substances.

In the present study:

Serum ferritin level was found to be significantly higher in the diseased groups when compared with control group.

On the other hand no differences were found among the diseased groups when compared with control group regarding both serum iron and serum total iron binding capacity levels.

Both serum superoxide dismutase and plasma thiobarbituric acid reactive substances were significantly higher in the diseased groups when compared with control group.

The result of this work showed a significant increase in serum total cholesterol, low density lipoprotein cholesterol in the diseased groups more than the control group. Also high density lipoprotein cholesterol was significantly lower in the diseased groups of patients when compared with the control.

Serum triglycerides level was significantly higher in the total group only when compared with control group in this study.

Conclusions:

1- Serum iron level is not critical in the process of lipid peroxidation. But serum ferritin is the determining factor being the source of iron.

2- The lipid peroxidation (as indicated by the thiobarbituric acid reactive substances) together with the increased atherogenic cholesterol both total, low density lipoprotein cholesterol and triglycerides all showed higher values in the diseased group.

3- As a result of the increased oxidative stress reported in the group of patients, Superoxide dismutase a component of antioxidant counterparts showed a reactive increase in the same groups.

REFERENCES

REFERENCES

1- Forrester J, Litvac F and Grundfest W. A perspective of coronary disease seen through the arteries of living man. Circulation 1987; 75: 505-13.

2- Baim DS, Harrison DC. Non atherosclerotic heart disease. In: The heart , arteries and veins Hurst JW, Longue RB, Rackley CG, Schalant RC, Sonnenblick EH, Walance AG, Wenger NK, eds., 5th ed. New York: McGrow Hill Book 1982; 1158-70.

3- Ross R. Atherosclerosis. In: Cecil Textbook of medicine. Wyngaarden JB, Smith LH, Bennett JC (editors). 5th ed. Philadelphia, London, Toronto: WB Saunders Company, 1992; 293-8.

4- Mario D, Schlant R. Coronary atherosclerosis. In: The heart. Hurst JW. New York, McGrow Hill Book company 1978; 1103-20.

5- Genest J, Cohn J. Clustering of cardiovascular risk factors: Targeting high-risk individuals. Am J of Cardiology 1995; 46(2): 8-20(A).

6- Bissett J, Wyeth R, Matts J, Johnson J. Plasma lipid concentrations and subsequent coronary occlusion after a first myocardial infarction. Am J Med Sci 1993; 305(3): 139-44.

7- Kannel WB. Cholesterol and risk of CHD and mortality in men. Clin Chem 1988; 34(8): 53-9(b).

8- Mezzetti A, Lapenna D, Pierdomenico S, Calafiore A, Costantini F. Vitamin E, C and lipid peroxidation in plasma and arterial tissue of smokers and non-smokers. Atherosclerosis 1995; 112: 91-9.

9- Frei B, Forte T, Ames B, Cross C. Gas phase oxidants of cigarrette smoke induce lipid peroxidation and changes in lipoproteins in human blood plasma. Biochem J 1991; 277: 133.

10- Scheffer E, Wiest E, Woehrle J. Smoking influences the atherogenic potential of LDL. Clin Invest 1992; 70: 263.

11- Chen C, Loo G. Cigarrette smoke extract inhibit oxidative modification of LDL. Atherosclerosis 1995; 112: 177-85.

12- Ross R. Diseases of blood vessels. In: Basic pathology. Robbins KC. 5th ed. Philadelphia, London, Toronto, Tokyo: WB Saunders Company, 1992; 227-304.

13- O'Keefe J, Lavie C, McCallister B. Insights into the pathogenesis and prevention of CHD. Mayo Clin Proc 1995; 70: 69-79.

14- Fallon JT. Pathology of myocardial infarction and reperfusion. In: Atherosclerosis and coronary artery disease. Fuster V, Ross R and Topol E J (eds). Philadelphia, Lippincott-Raven 1996; pp. 791-796.

15- Constantinides P. Plaque fissures in human coronary thrombosis. J Athero Res 1966; 6: 1.

16- Davies MJ and Thomas AC. Plaque fissuing- the cause of acute myocardial infarction, sudden ischaemic death, and crescendo angina. Br Heart J; 1985; 53: 363.

17- Flak E. Coronary thrombosis: Pathogenesis and clinical manifestations. Am J Cardiol 1991; 68: 28B.

18- Flak E, Shah PK and Fuster V. Pathogenesis of plaque disruption. In : Fuster V, Ross R and Topol EJ (eds.) atherosclerosis and coronary artery disease. Philadelphia, Lippincott-Raven 1996; pp. 492-510.

19- Willerson JT. Conversion from chronic to acute coronary heart disease syndromes: Role of platelets and platelet products. Tex Heart Inst J 1995; 22 : 13.

20- Flak E, Shah PK and Fuster V. Coronary plaque disruption. Circulation 1995; 92: 657.

21- Davies MJ, Richardson PD, Woolf N et al. Risk of thrombosis in human atherosclerotic plaques: Role of extracellular lipid, macrophage and smooth muscle cell content. Br Heart J 1993; 69: 377.

22- Kragel AH, Reddy SG, Wittes JT et al. Morphometric analysis of the scomposition of atherosclerotic plaques in the four major epicardial coronary arteris in acute mycocardial infarction and in sudden coronary death. Circulation 1989; 80:1747.

23- Roberts WC. Preventing and arresting coronary atherosclerosis. Am Heart J 1995; 130: 580.

24- Stary HC. The histological classification of atherosclerotic lesions in human coronary arteries. In: Fuster V, Ross R and Topol EJ (eds.). Atherosclerosis and coronary artery disease. Philadelphia, Lippincott-Raven 1996; 463-474.

25- Libby P. Molecular basis of the acute coronary syndromes. Circulation 1995; 91: 2844.

26- Falk E. Plaque rupture with severe pre-existing stenosis precipitating thrombosis: Characteristics of coronary atheroscelerotic plaque under lying fatal occlusion thrombi. Br Heart J 1983; 50: 127.

27- Wilson RF, Holida MD and Whie CW. Quantitative angiographic morphology of coronary stenoses leading to myocardial infarction or unstable angina. Circulation 1986; 73: 286.

28- Weiss EJ, Bray PF, Schulman SP et al. Fibrinogen receptor polymorphism PLA_2: An inherited platelet risk factor for early coronary thrombotic events. Circulation 1995; 92(suppl): 1-30.

29- Galis Z, Sukhova G, Lark M et al. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest 1994; 94: 2493.

30- Kovanen PT, Kaartinen J and Paavonen T. Infiltrates of activated mast cells at the site of coronary atheromatous erosion or rupture in myocardial infarction. Circulation 1995; 92: 1084.

31- Constantinides P. Infiltrates of activated mast cells at the site of coronary atheromatous erosion or rupture in myocardial infarction. Circulation. 1995; 92: 1083.

32- Balbier BM, Kipnes RS, Curnt JT. Biological defense mechanisms: The production of superoxide potential bactericidal agent. J Clin Invest 1973; 52: 741-744.

33- Simpson JA, Narita S, Gieseg S, Gebicki JM, Dean RT. Long-lived reactive species on free-radical-damaged proteins. Biochem J 1992; 282: 621-624.

34- Porter NA. Autoxidation of polyunsaturated fatty acids: Initiation, propagation and product distribution (basic chemistry) In: Vigo-Pelfery C, ed. Membrane lipid oxidation. Boca Raton: CRC, 1990; 33-62.

35- Kasai H, Nishimura S. Formation of 8-hydroxyguanosine in DNA by oxygen radicals and its biological significance. Sies H ed. Oxidative stress: Oxidants and antioxidants. London: Academic Press, 1991; 99-116.

36- Sangstad D. Neonatal oxygen radical disease. In David T. J Recent Advances in Paediatrics. Churchill Livingstone, Edinburgh, London, Melbourne 1992; 173-187.

37- Cheeseman KH and Slater TF. Free Radical in Medicine. British Medical Bulletin. 1993; 49(3): 479-723.

38- Halliwell B, Gutteridge JMC, Cross CE. Reactive oxygen species and human disease. J Lab Clin Med 1992; 119: 598-620.

39- Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease: An overview In: Packer L, Glazer AN (eds). Methods in Enzymology, vol. 186. San Diego, Academic Press, 1990; 1-85.

40- Halliwell B, Gutteridge JMC and Cross CE. Free radicals, antioxidants and huma disease: Where are we now? J Lab Clin Med 1992; 119(6): 598-620.

41- Saran M, Michel C, Bors W. Reactions of NO with O_2. Implications for the action of endothelium-derived relaxing factor. Free Radic Res Commun 1989; 83: 1705-1715.

42- Implay JA, Fridovich I. Assays of metabolic syperoxide production in Escherichia coli. J Biol Chem 1991; 266: 6957-65.

43- Madison DV. Pass the nitric oxide. Proc Natl Acad Sci USA 1993; 90: 4329-31.

44- Blake DR, Allen RE, Luvec J. Free radicals in biological systems - a review oriented to inflammatory processes. Br Med Bult 1987; 44(2): 371-385.

45- Esterbauer H, Cheeseman K. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxy nonenal. Methods Enzymol 1990; 186: 407-421.

46- Steinberg D. Lipoproteins and atherosclerosis: a look back and a look ahead. Arteriosclerosis 1983; 3: 283-310.

47- Tyroler HA. Lowering Plasma cholesterol levels decreases risk of coronary heart disease: an overview of clinical trials. In : Steinberg D, Olefsky JM (eds). Hypercholesterolaemia and atherosclerosis. New york :Churchill livingstone 1987;99-116.

48- Goldstein JL, Brown MS. The low-density lipoprotein pathway and its relation to atherosclerosis. Annu Rev Biochem 1977;46:897-930.

49- Newman HAI, Zilversmit DB. Quantitative aspects of cholesterol flux in rabbit atheromatous lesions. J Biol Chem 1962;237: 2078 - 84.

50- Fowler S, Shio H, Haley NJ. Characterization of lipid - laden aortic cells from cholesterol-fed rabbits. IV. Investigation of macrophage- like properties of aortic populations. Lab Invest 1979; 41: 372-8.

51- Rosenfeld ME, Tsukada T, Gown AM, Ross R. Fatty streak initaition in watanabe Heritable Hyperlipemic and comparably hypercholesterolemic fat-fed rabbits. Arteriosclerosis. 1987;1:9-23.

52- Wissier RW. Progression and regression of atherosclerotic lesions. Adv Exp Med Biol 1978; 104: 77-109.

53- Murno JM, Cotran RS. The pathogenesis of atherosclerosis: atherogenesis and inflammation. Lab Invest 1988; 58: 249-61.

54- Ross R. Atherosclerosis. In: McGee J, Isaacson PG, Wright NA. Oxford Textbook of Pathology. Vol. 2a. Oxford, New York, Tokyo: Oxford University Press. 1992; 798-812.

55- Brown MS, Goldstein JL. Lipoprotein metabolism in the macrophage: Implications for cholesterol deposition in atherosclerosis. Annu Rev Biochem 1983; 52: 223-61.

56- Buja LM, Kita T, Coldstein JL, Watanabe Y. Brown MS. Cellular pathology of progressive atherosclerosis in the WHHL rabbit: an animal model of familial hypercholesterolaemia. Arteriosclerosis 1983; 3: 87-101.

57- Kenriken T, Mahoney EM, Steinberg D. Enhanced macrophage degradation of low density lipoprotien previously incubated with cultrued endothelial cells: recognition by receptor for acetylated low density lipoproteins. Proc Natl Acad Sci USA 1981; 78: 6499-503.

58- Ide M. Enhanced macrophage degradation of biologically modified low density lipoprotein. Arteriosclerosis 1983; 3: 149-59.

59- Cathcart MK, Morel DW, Chisolm GM III. Monocytes and neutrophils oxidize low density lipoproteins making it cytotoxic. J Leukocyte Biol 1985; 38: 341-50.

60- Hirawatsu K, Rosen H, Heinecke JW, Wolfbauer G, Chait A. Superoxide initiates oxidation of low density lipoprotein by human monocytes. Arteriosclerosis 1987; 7: 55-60.

61- Steinbrecher UP, Parthasarathy S, Leake DS, Witztum JL, Steinberg D. Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. Proc Natl Acad Sci USA 1984; 83: 3883-7.

62- Steinbrecher UP, Witztum JL, Parthasarathy S, Teinberg D. Decrease in reactive amino groups during oxidation or endothelial cell modification of LDL: Correlation with changes in receptor-mediated catabolism. Arteriosclerosis 1987; 1: 135-43.

63- Fong LG, Parthasaratly S, Witztum JL, Steinberg D. Nonenzymatic oxidative cleavage of peptide bonds in apoportein Bio. J Lipid Res 1987; 28: 1466-77.

64- Movel DW, Dicarleto PE, Chisolm GM III. Endothelial and smooth muscle cells alter low-density lipoproteins making it cytotoxic. J Leukocyte Biol 1985; 38: 341.

65- Ross R, Glomset JA. The pathogenesis of atherosclerosis. N Engl J Med 1976; 295: 369-77.

66- Davies PF, Reidy MA, Goode TB, Bowyer DE. Scanning electron microscopy in the evaluation of endothelial integrity of the fatty lesion in atherosclerosis. Atherosclerosis 1976; 25: 125-30.

67- Fielding CI and Fielding PE. Cholesterol transport bẽtween cells and body fluids. Med Clin Nor Am 1982; 66(2): 363-366.

68- Glomsel JA and Norum KR. The metabolic role of lecithin cholesterol acyl transferase: Prespectives from pathology. Adv Lipid Res 1977; 11: 1.

69- Bachorik PS, Levy LI, Rifkind BM. Lipids and dyslipoproteinaemia. In: Henry JB (editor). Clinical diagnosis and management by laboratory methods. Volume 1. 18th edition. Philadelphia, London, Toronto: WB Saunders Company 1991; 188-214.

70- Stein EA, Myers GL. Lipids, apolipoproteins and lipoproteins. In: Tietz NW (editor). Fundamentals of Clin Chemistry. 4th edition. Philadelphia, London, Toronto: WB Saunders Company. 1996; 375-401.

71- Fielding PE, Fielding CJ. Acholesteryl ester transfer complex in human plasma. Proc Nati Acad Sci USA 1980; 77: 3327-3330.

72- Goldstein JL and Brown MS. Regulation of low density lipoprotein receptors: Implications for pathogenesis and therapy of hypercholesterolaemia and atherosclerosis. Circulation 1987; 76(3): 504-507.

73- Mahley RW. Atherogenic hyperlipoproteinaemia: The cellular and molecular biology of plasma lipoproteins altered by dietary fat and cholesterol. Med Clin Nor Am 1982; 66(2): 375-402.

74- Kasaniemi YA, Grundy SM. Moderate hypercholesterolaemia is caused by over production of LDL. Clin Res 1981; 29: 541.

75- Grundy SM. Hypertriglyceridaemia mechanisms: Clinical significance and treatment. Med Clin Nor Am 1982; 66(2): 519-535.

76- Brunzell JD, Schrott HG, Motulsky AG. Myocardial infarction in familial forms of hypertriglyceridaemia. Metabolism 1976; 25: 313.

77- Brunzell JD, Bierman EL. Chylomicronemia syndrome: Interaction of genetic and aquired hypertriglyceridaemia. Med Clin Nor Am 1982; 66(2): 455-468.

78- Breckenridge WC, Little JA, Steiner G. Hypertriglyceridaemia associated with deficiency of apoliporprotein C-II. N Engl J Med 1978; 298: 1265-1273.

79- Shrrill BC, Innerarity TL, Mahley RW. Rapid hepatic clearance of the canine lipoproteins containing only the E apoprotein by a high affinity receptor: Identity with the chylomicron remnant transport process. J Biol Chem 1980; 255: 1804-1807.

80- Goldstein JL and Brown MS. The LDL receptor defect in familial hypercholesterolaemia: Implications for pathogenesis and therapy. The Med Clin Nor Am 1982; 66(2): 335-362.

81- Halliwell B. Free radicals, reactive oxygen species and human disease: A critical evaluation with special reference to atheroslerosis. Br J Exp Path 1989; 70: 737-57.

82- Beutler B, Fairbanks VF, Fahey JL. The metabolism of iron. In Clinical Disorders of Iron Metabolism. New York, Grune & Stratton 1963: 39.

83- Kennedy MC, Mende-Mueller L and Blondin GA. Purification and characterization of cystosolic aconitase from beff liver and its relationship to the iron responsive element binding protein. Proc Natl Acad Sci USA 1992; 89: 11730.

84- Burtis CA, Ashwood ER. Tietz Fundamentals of Clinical Chemistry. W.B. Saunders Company. A division of Harcourt Brace Company. Philadelphia, London, Toronto, Montreal, Sydney, Tokyo. 1996; 2: 727.

85- Morgan EH. Transferrin, biochemistry, physiology and clinical significance. Mol Aspects Med 1981; 4: 1.

86- MacGillivary RTA, Mendez E, Shewale JG. The primary structure of human transferrin: The structure of seven cyanogen bromide fragments and the assembly of the complete structure. J Biol Chem 1983; 258: 3543.

87- Cazzola M, Huebers HA, Sayers MH, Macphail P, Eng M, Finch CA. Transferrin saturation, plasma iron turnover and transferrin uptake in normal humans. Blood 1985; 66: 935-939.

88- Qiau ZM, Morgan EH. Changes in the uptake of transferrin free and trasnferrin bound iron during reticulocyte maturation in vivo and in vitro. Bio Phys Acta 1992; 1135: 35.

89- Pootrakul P, Josephson B, Huebers HA. Quantitation of ferritin iron in plasma an explanation for nontrasnferrin iron. Blood 1988; 71: 1120-1125.

90- Worwood M. Serum ferritin. Clinical Science 1986; 70: 215-220.

91- Boyd D, Vecoli C, Belcher DM. Structural and functional relationships of human ferritin H and L chains deduced form cDNA clones. J Biol Chem 1985; 260: 11755.

92- Stefani S, Sesideri A, Vecchini P. Identification of the iron entery channels in apoferritin: Chemical modification and spectrophotometric studies. Biochemistry 1989; 28: 378-383.

93- Wardeska JG, Viglione B and Chasteen ND. Metal ion complex of apoferritin: Evidence for initial binding in the hydrophilic channels. J Biol Chem 1986; 261: 6677.

94- Oconnell MJ, Ward RJ, Baum H. The role of iron in ferritin and haemosidrin-mediated lipid peroxidation in liposomes. Bio Chem J 1985; 229: 135-141.

95- Sies H. Oxidative stress: Introductory remarks: In oxidative stress. Edited by Sies H, Orlando FL Academic Press 1985: 1-8.

96- Wills ED. The role of dietary components in oxidative stress in tissues. Edited by Sies H, Orlando FL Academic Press 1985; 197-220.

97- Witz G. Biological interactions of alpha, beta-unsaturated aldehydes. Free Radic Biol Med 1989; 7: 333-349.

98- Dascoe GA, Reed DJ. Cell calcium, vitamin E and the ethiolredox system in cytotoxicity. Free Radic Biol Med 1989; 6: 209-224.

99- Brot H, Weissbach H. Biochemistry and physiological role of methionine sulphoxide residues in proteins. Arch Biochem Biophys 1983; 223: 271-281.

100- Fridovich IJ. Superoxide Dismutases: An adaptation to a paramagnetic gas. J Biol Chem 1989; 264: 7761-7764.

101- Stonz G, Tartaglia LA, Ames BN. Transcriptional regulator of oxidative stress-inducible Genes: Direct activation by oxidation. Science 1990; 248: 189-194.

102- Marlaund SL. Human copper – containing superoxide dismutase of high molecular weight. Proc Natl Acad Sci USA 1982; 79: 7634-7638.

103- Jones DP. Intracellular catalse function: Analysis of the catalytic activity by product formation in isolated liver cells. Archiv Biochem Biophys 1982; 214: 806-814.

104- Greenwald RA. Superoxide dismutase and catalase as theraputic agents for human diseases. Free Radic Biol Med 1990; 8: 201-209.

105- Stocker R, Glazer AN, Ames BN. Antioxidant activity of albumin-bound bilirubin. Proc Natl Acad Sci USA 1987; 84: 5918-5922.

106- Steinbrecher UP, Zhang H, Lougheed M. Role of oxidatively modified LDL in atherosclerosis. Free Radic Biol Med 1990; 9: 155-168.

107- Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol: Modification of low density lipoprotein that increases its atherogenecity. N Engl J Med 1989; 320: 915.

108- Halliwell B, Gutteridge JMC. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J 1984; 219: 1.

109- Sparrow CP, Olazewski J. Cellular oxidation of low density lipoprotein is caused by thiol production in mediating transition metal ions. J Lipid Res 1993; 34: 1219-1228.

110- Aust SD, Morehouse LA, Thomas CE. Role of metals in oxygen radical reactions. J Free Radicals Biol Med 1985; 1: 3-25.

111- Halliwell B, Gutteridge JMC. Protection against tissue damage in vivo by desferrioxamine: What is its mechanism of action? J Free Radicals in Biology and Medicine 1989; 7(6): 645-651.

112- Ganner B, Dean RT, Jessup W. Human macrophage- mediated oxidation of low density lipoprotein is delayed and independent of superoxide production. Bioch J 1994; 301: 421-428.

113- Carlin G, Djursater R. Xanthine oxidase induced by polymerization of hyaluronic acid in the presence of ferritin. FEBS Lett 1984; 177: 27-30.

114- Steinbrecher UP. Oxidation of human low density lipoprotein results in derivatization of lysine residues of Apolipoprotein B by lipid peroxide decomposition products. J Biol Chem 1987; 262: 3603-3608.

115- Steinbrecher UP, Lougheed M, Kwan WC, Dirk SM. Recognition of oxidized low density lipoprotein by the scavenger receptor of macrophages results from derivatization of Apolipoprotein B by products of fatty acid peroxidation. J Biol Chem 1989; 264: 15216-15223.

116- Vile GF, Winterbourn CC. Iron bindin to microsomes and liposomes in relation to lipid peroxidation. FEBS Lett 1987; 215: 151-154.

117- Varley H, Gowenlock AH, Bell M. Practical Clinical Biochemistry volume I. General topics and commoner tests. 6th ed. Reset, London: William Heinmann Medical Books Ltd, 1988; 321-4.

118- Dacie JV, Lewis SM. Practical haematology. 8th ed. Edinburgh, London: Churchill Livingstone, 1995; 37-73.

119- Trinder P. Ann Clin Biochem 1969; 6: 24 (Quoted from Varley H, Gowenlock AH and Bell M, Practical Clinical Biochemisty 5th ed. Heinmann Medical Books Ltd London 1980; 1: 405.

120- Sampson EJ, Baird MA, Burtis CA, Smith EM, Wilte DL, Bayse DD. A coupled enzyme equilibrium for measuring urea in serum: Optimization and evaluation of the AACC study on urea: Candidate reference method. Clin Chem 1980; 26: 816-826.

121- Henry JB, Nelson DA, Tomar RH, Washington JA, Threatte GA. Clinical diagnosis and management by laboratory methods. 18th ed., WB Saunders Company. Philadelphia, London, Toronto, Montreal, Sydney, Tokyo 1991; 1: 143.

122- Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total cholesterol. Clin Chem 1974; 20: 470-475.

123- Henry JB, Nelson DA, Tomar RH, Washington JA, Threatte GA. Clinical diagnosis and management by laboratory methods. 18th ed., WB Saunders Company. Philadelphia, London, Toronto, Montreal, Sydney, Tokyo 1991; 1: 197.

124- Burstein M, Scholnich HR and Morfin R. Rapid method for isolation of lipoproteins from human serum by precipitation with polyanions. J Lipid Res 1970; 11: 563-595.

125- Waston D. Estimation of B-lipoproteins, colourimetric method. Clin Chem Acta 1960; 5: 37.

126- Bernhiem F, Bernhiem MLC, Wilbur KM. The reaction between thiobarbituric acid and the oxidation products of certain lipids. J Biol Chem 1984; 174: 257.

127- Lamb DJ, Wilkins GM, Leaks DS. The oxidative modification of low density lipoprotein by human lymphocytes. Atherosclerosis 1992; 92: 187-192.

128- Engvall E. Methods in Enzymology. Van Vunakis H, Langone JJ, Editors. New York: Academic Press 1980; 70: 419-92.

129- Cerriti F and Cerriti G. Improved direct specific determination of serum iron. Clin Chem 1980; 26(2): 327-31.

130- Marklund S and Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974; 47: 469-474.

131- Knapp RG, Miller MC. Clinical Epidemiology and Biostatistics. Harwal Publishing company. Pennsylvania (Egyptian Edition, Mass Publishing Company) 1992; 255-92.

132- Aust SD and Svingen BA. The role of iron in enzymatic lipid peroxidation. Free Radicals Biol 1982; 5: 1-28.

133- McCord JM. Is iron sufficiency a risk factor in ischemic heart disease? Circulation 1991; 83: 1112-1114.

134- Cross CE, Halliwell B, Borish ET, Pryor WA, Saul RL, McCord JM, Harman D. Oxygen radicals and human disease. Ann Intern Med 1987; 107: 526-545.

135- Samokyszyn VM, Reif DW, Miller DM, Aust SD. Effect of ceruloplasmin on superoxide-dependent iron release from ferritin and lipid peroxidation. Free Radic Res Commun 1991; 12: 153-159.

136- Balla G, Jacob HS, Eaton JW, Beicher JD, Vercellotti GM. Hemin: A possible physiological mediator of low density lipoprotein oxidation and endothelial injury. Arterioscler Thromb 1991; 11: 1700-1711.

137- Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol: Modification of low density lipoprotein that increase its atherogenicity. N Engl J Med 1989; 320: 915-923.

138- Kuzuya M, Naito M, Yamada K, Funaki C, Hayashi T, Asai K, Kuzuya F. Involvement of intracellular iron in the toxicity of oxidized low density lipoprotein to cultured endothelial cells. Biochem Int 1990; 22: 567-573.

139- Wilkins GM, Leake DS. Free radicals and low density lipoprotein oxidation by macrophages. Biochem Soc Trans 1990; 18: 1170-1171.

140- Buja LM, Roberts WC. Iron in the heart: Etiology and clinical significance. Am J Med 1971; 51: 209-21.

141- Leon MB, Boret JS, Bacharach SL, et al. Detection of early cardiac dysfunction in patients with early cardiac dysfunction in patients with severe beta-thalassemia and chronic iron overload. N Engl J Med 1979; 301: 1143-48.

142- Vigorita VJ, Hutchins GM. Cardiac conduction system in Hemochromatosis: Clinical and pathologic features of six patients. Am J Cardiol 1979; 44: 418-23.

143- Richter GW. The iron laoded cell – the cytopathology of iron storage. A review Am J Path 1978; 91: 362-404.

144- Sullivan JL. The sex difference in ischemic heart disease. Perspect Biol Med 1983; 26: 657-71.

145- Gannon DE, Varani J, Phan SH, Ward JH, Kaplan J, Till GO, Simon RH, Ryan US, Ward PA. Source of iron in neutrophil – mediated killing of endothelial cells. Lab Invest 1987; 57: 37-44.

146- Heinecke JW, Rosen H, Chait A. Iron and copper promote modification of low density lipoprotein by human arterial smooth muscle cells in culture. J Clin Invest 1984; 74: 1890-4.

147- Heinecke JW, Baker L, Rosen H, Chait A. Superoxide mediated modificiation of low density lipoprotein by arterial smooth muscle cells. J Clin Invest 1986; 77: 757-61.

148- Salonen JT, Nyyssonen K, Korpela H, Tuomilehto J, Seppaven R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern finnishmen. Circulation 1992; 86: 803-11.

149- Rimm EB, Ascherio A, Stampfer MJ, Colditz GA, Giovannucci E, Wille WC. Dietary iron intake and risk of coronary disease among men. Circulation 1993; 87: 692. Abstract.

150- Cooper RS, Liao Y. Iron stores and coronary heart disease: negative findings: The NHANES I Epidemiologic follow-up study. Circulation 1993; 87: 680. Abstract.

151- Christopher T, Anne C, Richard F, Diane M. Body iron stores and the risk of coronary heart disease. The new England Journal of Medicine 1994; 330: 1119-1124.

152- Worwood M. Ferritin in human tissues and serum. Clin Hematol 1982; 11: 275-307.

153- Konijn AM. Iron metabolism in inflammation. Baillieres Clin Haematol 1994; 7: 829-49.

154- Finlayson J, Fraser CG. Short term changes in iron, ferritin, total iron binding capacity and transferrin in serum after myocardial infarction. Clin Chem 1985; 31: 782-3.

155- Cavanna F, Ruggeri G, Iacobello C, Chiergatti G, Murador E, Albertini A, Arosio P. Development of a monoclonal antibody

against human heart ferritin and its application in an immunoradiometric assay. Clin Chim Acta 1983; 134: 347-56.

156- Fillet G, Cook JD, Finch CA. Storage iron kinetics: A biological model for reticuloendothelial iron transport. J Clin Invest 1974; 53: 1527-33.

157- Birgens HS. The monocytic receptor for lactoferrin and its involvement in lactoferrin-mediated iron transport. Adv Exp Med Biol 1994; 357: 99-109.

158- Niki E. Antioxidant defenses in eukaryocytic cells. In: Free radicals: From Basic Science to Medicine. Poli G, Albano E and Dianzani MU, eds. Basel, Switzerland: Birkhauser Verlag, 1993; 365-73.

159- MacCard JM. Iron, free radicals and oxidative injury. Seminars in haematology 1998; 35(1): 5-12.

160- Sullivan JL. Iron and sex difference in heart disease risk. Lancet 1981; 1: 1293.

161- Stampfer MJ, Grodstein F, Rosenberg I, Willett W, Hennekens C. A prospective study of plasma ferritin and risk of myocardial infarction in US physicians. Circulation 1993; 87: 688.

162- Haberland ME, Fogelman AM. The role of altered lipoproteins in the pathogenesis of atherosclerosis. Am Heart J 1987; 113: 573.

163- Parthasarathy S, et al. Essential role of phosphlipase A_2 activity in endothelial cell-induced modification of low density lipoprotein. Proc Natl Acad Sci USA 1985; 82: 3000.

164- Haberland ME, et al. Role of lysines in mediating interaction of modified low density lipoproteins with the scavenger receptor of human monocytes macrophages. J Biol Chem 1984; 259: 11305.

165- Yong W, Xian XQ, Liang GJ, Bin SG, Fei YJ, Yun DH, Fang SH. Correlation of serum lipids, lipoproteins, lipid peroxide products and metals with coronary heart disease. Chinese Medical Journal 1993; 106(3): 167-170.

166- Calabresi L, Franceschini G, Sirtori M, Gianfranceschi G. Influence of serum triglycerides on the HDL pattern in normal subjects and patients with CAD. Atherosclerosis 1990; 84: 41-8.

167- NIH Consensus Development Conference. Triglyceride, HDL and CHD. JAMA 1993; 269(4): 505-10.

168- Cheeseman KH, Clater TS. Free radicals in Medicine. British Medical Bulletin 1993; 49(3): 679-723.

169- White CW, Avaham KB, Shanley PF, Groner Y. Transgenic mice with expression of elevated levels of Cu/Zn SOD in the lungs are resistant to pulmonary oxygen toxicity. J Clin Invest 1991; 87: 2162-8.

170- Accord LM, Fridovich I. Superoxide dismutase: an enzyme function for erythrocuprein (hemocuprein). J Biol Chem 1969; 244: 6049-55.

171- Accord LM, Keele BB Jr, Fridovich I. An enzyme-based theory of obligate anaerobiosis: The physiological function of superoxide dismutase. Proc Natl Acad Sci USA 1971; 68: 1024-7.

172- Weisiger RA, Fridovich I. Mitochondria superoxide dismutase. Site of synthesis and intramitochondrial localization. J Biol Chem 1973; 248: 4793-6.

173- Fridovich I. Superoxide dismutase. Annu Rev Bio Chem 1975; 44: 147-59.

174- Marklund SL, Westman NG, Lundgrem E, Roos G. Copper and zinc – containing superoxide dismutase, catalase, and glutathione peroxidase in normal and neoplastic human cell lines and normal human tissues. Cancer Res 1982; 42: 1955-61.

175- Barra D, Schinina M, Simmaco M, et al. The primary structure of human liver manganese superoxide dismutase. J Biol Chem 1984; 259: 12595-601.

176- Markland SL. Superoxide dismutase in human tissues, cells and extracellular fluids: Clinical implications. In: Johnson JE, eds. Free radicals, aging and degenerative disease. New York: Alan R Liss 1986; 509-26.

177- Slot JW, Geuze HJ, Freeman BA, Crapo JD. Intracellular localization of the Cu, Zn and Mn-superoxide dismutase in rat liver parenchymal cells. Lab Invest 1986; 55: 363-71.

178- Bjerrum MJ. Structural and spectroscopic comparison of manganese – containing superoxide dismutases. Biochem Biophys Acta 1987; 915: 225-37.

179- Usui A, Kato K, Tsuboi H, Sone T, Sassa H, Abe T. Concentration of Mn-superoxide dismutase in serum in acute myocardial infarction. Clin Chem 1991; 37/3: 458-461.

PROTOCOL

STUDY OF SERUM IRON AND FERRITIN IN PATIENTS WITH ISCHEMIC HEART DISEASES

دراسة مستوى الحديد والفيريتين في مصل مرضى قصور الشرايين التاجية

Protocol of thesis submitted
to Medical Research Institute
Alexandria University
In partial fulfillment of
Master Degree

In Chemical Pathology

خطة بحث مقدمة
لمعهد البحوث الطبية
جامعة الإسكندرية
إيفاءاً جزئياً للحصول على
درجة الماجستير

في الباثولوجيا الكيميائية

By

Samah Saad Abd El-Aleem
M.B.B.Ch
(Alex.) 1992

من الطبيبة

سماح سعد عبد العليم
بكالوريوس الطب والجراحة
الإسكندرية ١٩٩٢

1998

١٩٩٨

SUPERVISORS

المشـــــــرفون

Prof. Dr. Ahmad Mohamad Zaki

Professor & Head of
Chemical Pathology Department
Medical Research Institute
Alexandria University

أ.د. أحمد محمد زكى

أستاذ ورئيس قسم
الباثولوجيا الكيميائية
معهد البحوث الطبية
جامعة الإسكندرية

Dr. Yousri I. Ahmed

Assistant Professor of Chemical Pathology
Medical Research Institute
Alexandria University

د. يسرى إسماعيل أحمد

أستاذ مساعد الباثولوجيا الكيميائية
معهد البحوث الطبية
جامعة الإسكندرية

Dr. Amel Abd El Fattah Kamel

Lecturer of Chemical Pathology
Medical Research Institute
Alexandria University

د. أمل عبد الفتاح كامل

مدرس الباثولوجيا الكيميائية
معهد البحوث الطبية
جامعة الإسكندرية

Dr. Kamal Mahmoud Ahmed

Lecturer of Internal Medicine
Medical Research Institute
Alexandria University

د. كمال محمود أحمد

مدرس الأمراض الباطنية والقلب
معهد البحوث الطبية
جامعة الإسكندرية

INTRODUCTION

The first step in atheroma formation is LDL oxidation,[1] the oxidized LDL is taken by phagocytic cells to form what is termed as "foam cells": the cholesterol loaded macrophage-derived cells of the atherosclerotic lesion.[2]

Oxidized LDL is taken up by macrophages via scavenger receptors, leading to cholesterol accumulation.[3]

Native LDL, however, is not recognized by scavenger receptors and thus does not cause cholesterol accumulation in macrophages.[4]

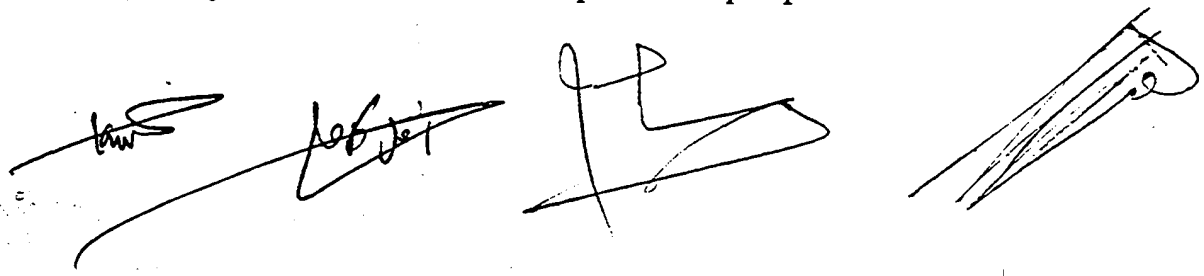
Monocytes, the precursors of macrophages are attracted towards oxidized LDL by chemotaxis and therefore oxidized LDL may attract monocytes into subendothelial space.[5]

It has been shown that LDL modification is due to "free radical reaction" and that both copper and iron in reduced forms are able to induce such process.[6]

To promote free radical reaction, iron is required promoting lipid peroxidation according to Fenton reaction.

$$H_2O_2 + FeII \rightarrow OH^- + OH^\bullet + FeIII$$ [7]

This reaction liberates highly reactive oxygen species which is "hydroxyl radical" $OH^\bullet$ that is capable of lipid peroxidation.[8]



It has been reported by some workers that increased level of plasma ferritin is associated with increase incidence of myocardial atherosclerosis due to increased LDL oxidation.[9]

AIM OF THE WORK

The aim of the present work is to study serum iron and ferritin in a trial to evaluate their possible role in coronary atherosclerosis.

MATERIAL AND METHODS

Material

Thirty subjects will be included in the study. They will be divided as follows:-

1- Fifteen patients with stable angina.

2- Fifteen patients with unstable angina.

2- Ten apparently normal healthy control subjects.

All subjects will be of comparable age, sex and socio-economic status.

Methods

To all studied subjects:

1- Thorough history taking and assessment of risk factors profile.

2- Full clinical examination.

3- Standard twelve lead ECG.

4- The following laboratory investigations will be done:

- Urine analysis.[10]
- From fasting patients (at least 12 hours) blood sample will be drawn for estimation of the following:-
- Complete blood picture.[11]
- Fasting blood sugar.[10]

- Serum urea.[10]
- Serum creatinine.[10]
- Total serum cholesterol.[10]
- High density lipoprotein cholesterol.[12]
- Low density lipoprotein cholesterol.[13]
- Serum triglycerides.[10]
- Thiobarbituric acid reactive substances (TBARs).[14]
- Serum iron, ferritin, total iron binding capacity.[15]
- Serum superoxide dismutase (SOD).[16,17]

RESULTS AND DISCUSSION

The results obtained from the study will be tabulated statistically, analyzed, compared with other available works and discussed.

REFERENCES

1- Steinberg D, Parthasarathy S, Carew TE, Khoo IC, Witztum JL. Beyond cholesterol: modification of low density lipoprotien that increases its pathogenicity. N Engl J Med 1989; 320: 915.

2- Rosenfeld ME, Tsukada T, Gown AM, Ross R. Fatty streak initiation Watanabe Heritable Hyperlipemic and comparably hypercholesterolamic fat-fed rabbits. Arteriosclerosis 1987; 1: 9-23.

3- Goldstein JL, Brown MS. The low density lipoprotein pathway and relation to atherosclerosis. Annu Rev Biochem 1977; 46: 897-930.

4- Ideu. Enhanced macrophage degradation of biologically modified low-density lipoprotein. Arteriosclerosis 1983; 3: 149-59.

5- Quinn MT, Parthasarathy S, Fong LG, Steinberg D. Oxidatively modified low density lipoproteins: A potential role in recruitment and retention of monocyte/macrophages during atherogenesis. Proc Natl Acad Sc USA 1987; 84: 2995-8.

6- Halliwell B, Gutteridge JMC. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J 1984; 219: 1.

7- Ganner B, Dean RT, Jessup W. Human macrophage-mediated oxidation of low density lipoprotein is delayed and independent of superoxide production. Biochem J 1994; 301: 421-428.

8- Carlin G, Djursater R: Xanthine oxidase induced de polymerization of hyaluronic acid in the presence of ferritin. FEBS Lett 1984; 177: 27-30.

9- Moroz C, Bessler H, Katz M, Zahari I, Salman H, Djaldetti M: Elevated serum ferritin level in acute myocardial infarction. Biomedicine, Pharmacotherapy 1997; 51 (3): 126-30.

10- Varley H, Gowenlock AH and Bell M. Practical Clinical biochemistry. 5th ed. Vol I. London W. Heinemann Medical Books, Ltd 1980.

11- Dacie JV, Lewis SM. Practical hematology 4th ed. J and A Churchill Ltd London 1969.

12- Burstein M, Scholnich HR and Morfin R. Rapid method for isolation of lipoproteins from human serum by precipitation with polyanions. J Lipid Res 1970; 11: 563-595.

13- Watson D. Estimation of B. Lipoproteins, Colorimetric method. Clin Chim Acta 1960; 5: 37.

14- Bernhiem F, Bernhiem MLC and Wibur KM. The reaction between thiobarbituric acid and the oxidation products of certain lipids. J Biol Chem 1984; 174: 257.

15- Burtis C and Ashwood E. Tietz textbook of clinical chemistry 2nd edition. WB Saunders Company: Philadelphia - London 1994.

16- Bloc WJ, Li JY, Taylor PR et al. Nutrition intervention trials in Linxian, China: Supplementation with specific vitamin / mineral combinations, cancer incidence and disease specific mortality in the general population. J Natl Cancer Inst 1993; 85: 1483- 1492.

17- Saran M, Michel C, Bors W. Reactions of NO with O_2. Implications for the action of endothelium derived relaxing factor. Free Radical Res Commun 1989; 83: 1705-1715.

Arabic Summary

الملخص العربى

دراسة مستوى الحديد والفيريتين فى مصل مرضى

قصور الشرايين التاجية

يمثل التأكسد التحورى للدهنيات منخفضة الكثافه وكذلك مدى استيعابها بواسطة الخلايا الملتهمة خطوات مهمة فى حدوث تصلب الشرايين خاصة وأن هذه العملية لا تقل بدخول الكوليستيرول وأملاحه داخل الخلايا وبهذا يتم تكوين الخلايا الرغوية المشبعة بالدهون.

كما ثبت أن تحور الدهنيات المنخفضة الكثافة يعود إلى وجود الشوارد الحرة والتى قد تحدث نتيجة تواجد عنصرى الحديد والنحاس والأمر يتطلب تحرر الحديد من بروتيناته الحاملة له وهذا قد يحدث نتيجة توافر الحمل التأكسدى ذاته. وعلى المستوى النظرى فإن مادة الفيريتيين كمادة ضامة للحديد وعليه فإنه يمكن اعتبارها كمادة مضادة للأكسدة. ولكن الواقع يثبت العكس حيث أنه يمكن اعتبارها مخزنا ينبعث منه الحديد.

والغرض من هذه الرسالة هو دراسة مستوى الحديد والفيريتين فى مصل مرضى قصور الشرايين التاجيه فى محاولة لتقييم أثرهما المحتمل فى حدوث تصلب شرايين القلب.

وقد أجريت هذه الدراسة على ثلاثين مريض بمرض قصور الشرايين التاجية ممن دخلوا فى قسم الأمراض الباطنة بمعهد البحوث الطبية وقد تم تقسيم مجموعة المرضى إلى نصفين يتكون كل منهما من خمسة عشر مريضا ممن يعانون من مرض قصور الشرايين التاجية المستقر والغير مستقر هذا بالإضافة إلى مجموعة ضابطة مكونه من عشرة أشخاص طبيعيين.

وقد أجرى لكل شخص شملته هذه الدراسة فحص أكلينيكى كامل ورسم قلب كهربائى بالإضافة إلى الأبحاث المعملية الآتية: بول كامل – عد دم – سكر صائم – بولينا وكرياتينين – كولستيرول كلى ومرتفع ومنخفض الكثافة ودهون ثلاثية وحديد المصل

والفريتيين وكلا منّ انزيم فوق الأكسيد المانع للتحور SOD ومستوى المركبات المتفاعلة مع حمض الثيوباربيتيوريك TBARs.

وقد وجد أن:

١- مستوى الفيريتيين أظهر ارتفاعا ملحوظا فى مجموعات المرضى عن المجموعة الضابطة.

٢- لم تظهر فروق فى مستويات الحديد والقدرة الكلية لضم الحديد فى مصل جميع من شملتهم الرسالة.

٣- أظهر كل من ال SOD وال TBARs ارتفاعا ملحوظا فى المجموعات المرضية عن المجموعة الضابطة.

٤- أظهرت الدراسة ارتفاعا ملحوظا فى تركيز كل من الكولستيرول الكلى ومنخفض الكثافة والدهون الثلاثية فى المجموعات المرضية عن المجموعة الضابطة بينما انخفض مستوى الدهنيات المرتفعة الكثافة فى مجموعة المرضى.

وبهذا يمكن الوصول للنتائج الآتية:

١- إن مستوى حديد المصل لا يمثل أهمية كبرى فى عملية أكسدة الدهنيات بينما الفيريتيين هو العامل المؤثر.

٢- إن عملية أكسدة الدهنيات كما يستدل عليها من زيادة ال TBARs بالإضافة إلى الزيادة فى مستوى كل من الكولستيرول والدهنيات منخفضة الكثافة والدهون الثلاثية وكلها مؤشرات على تصلب الشرايين قد أظهرت ارتفاعا فى المجموعة المرضية.

٣- نتيجة لإرتفاع هذه المواد فإن مستوى ال SOD قد ازداد كرد فعل لمعادلة التأثيرات الضارة للمواد المؤكسدة.

المشرفون

أ.د/ أحمد محمد زكى

أستاذ ورئيس قسم الباثولوجيا الكيميائية

معهد الحبوث الطبية

جامعة الإسكندرية

د./ يسرى اسماعيل أحمد

أستاذ مساعد الباثولوجيا الكيميائية

معهد البحوث الطبية

جامعة الإسكندرية

د./ أمل عبد الفتاح كامل

مدرس الباثولوجيا الكيميائية

معهد البحوث الطبية

جامعة الإسكندرية

د./ كمال محمود أحمد

أستاذ مساعد الأمراض الباطنة والقلب

معهد البحوث الطبية

جامعة الإسكندرية

جامعة الاسكندرية

معهد البحوث الطبية

الدراسات العليا

موافقة العميد على تشكيل لجنة الحكم ١٢ / ٣ / ٢٠٠١

أعضاء اللجنة :

١- أ.د. أحمد محمد زكى أستاذ البايولوجيا الكيميائية بالمعهد (مشرف ومحكم داخلى)

٢- أ.د. منى محمد أباظة أستاذ البايولوجيا الكيميائية بالمعهد (محكم داخلى)

٣- أ.د. مصطفى زايد أستاذ مساعد البايولوجيا الكلينيكية بكلية الطب - جامعة الإسكندرية (محكم خارجى)

٤- أ.د.

موافقة مجلس المعهد على منح الدرجة ١٢ / ٦ / ٢٠٠١

يعتمد **

وكيل المعهد

للدراسات العليا والبحوث

١٢ / ٦

(أ.د. عزت محمد حسن)

دراسة مستوى الحديد والفيريتين فى مصل مرضى قصور الشرايين التاجية

رسالة مقدمة

لمعهد البحوث الطبية

ايفاء جزئيا للحصول على

درجة الماجستيرفى

الباثولوجيا الكيميائية

من

الطبيبة/ سماح سعد عبد العليم الشيخ

بكالوريوس الطب والجراحة

الإسكندرية (١٩٩٢)

معهد البحوث الطبية

جامعة الإسكندرية

٢٠٠١