"OXIDATIVE STRESS AND ANTIOXIDANT DEFENSE IN ACUTE MYOCARDIAL INFARCTION- A CASE CONTROL STUDY"

By

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Dissertation

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Under the guidance of
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DEPARTMENT OF BIOCHEMISTRY

JAWAHARLAL NEHRU MEDICAL COLLEGE,

BELGAUM-590010.

MAY - 2010

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LIST OF ABBREVIATIONS USED

AMI	-	Acute myocardial infraction
AST	-	Aspartate aminotransferase
apo-Lp	-	Apolipoprotein
CAD	-	Coronary artery disease
CRP	-	C- reactive protein
CT-I	-	Cardiac troponin I
CT-T	-	Cardiac troponin T
CK	-	Creatine phosphokinase
CCl_4	-	Carbon tetrachloride
$CCl_3^{\bullet}$	-	Trichloromethyl radical
CH_2	-	Methylene
Cu	-	Copper
DNA	-	Deoxy ribonucleic acid
D/W	-	Distilled water
Da	-	Dalton
DM	-	Diabetes mellitus
ECG	-	Electrocardiogram
Fe	-	Iron
Fe^{2+}	-	Ferrous iron
Fe^{3+}	-	Ferric iron
FAD	-	Flavin adenine dinucleotide
g or gm	-	Gram
GSSG	-	Oxidized glutathione
GSH	-	Reduced glutathione

GR	-	Glutathione reductase
GSH-Px	-	Glutathione peroxidase
HDL	-	High density lipoprotein
H_2O_2	-	Hydrogen peroxide
$HCOO^{\bullet}$	-	Hydroperoxyl radical
HOCl	-	Hypochlorus acid
HCl	-	Hydrochloric acid
Hb	-	Hemoglobin
H_2O	-	Water
IU	-	International units
IDL	-	Intermediate density lipoprotein
IHD	-	Ischaemic heart disease
Kg	-	Kilogram
LDH	-	Lactate dehydrogenase
LV	-	Left ventricle
LPL	-	Lipoprotein lipase
LCAT	-	Lecithin cholesterol acyl transferase
L	-	Litre
LDL	-	Low density lipoprotein
mmol	-	Milli mol
μmol	-	Micro mol
mg/dl	-	Milligram/deci litre
Min	-	Minute
Ml	-	Milli litre
Mn	-	Manganese

MPO	-	Myeloperoxidase
MDA	-	Malondialdehyde
$NO^{\bullet}$	-	Nitric oxide radical
NADPH	-	Nicotinamide adenine dinucleotide phosphate
NADH	-	Reduced nicotinamide adenine dinucleotide
nmol	-	Nano mol
$O_2CCl_3^{\bullet}$	-	Peroxyl radicals
$ONOO^-$	-	Peroxynitrite anion
O_2	-	Singlet oxygen
$OH^{\bullet}$	-	Hydroxyl radical
$O_2^{\bullet -}$	-	Superoxide anion radical
PUFA	-	Polyunsaturated fatty acid
PAI-1	-	Plasminogen Activator Inhibitor-1
PGI_2	-	Prostaglandin I_2
PDGF	-	Platelet derived growth factor
RBC	-	Red blood cells
$RS^{\bullet}$	-	Thiyl radical
$R^{\bullet}$	-	Lipid radical
$ROO^{\bullet}$	-	Lipid peroxide radical
RV	-	Right ventricle
RDA	-	Recommended daily allowance
ROS	-	Reactive Oxygen species
TBA	-	Thiobarbituric acid
TBAS	-	Thiobarbituric acid reactive substances
TAG	-	Triacyl glycerol

TXA_2	-	Thromboxane A_2
UV	-	Ultraviolet
VLDL	-	Very low density lipoprotein
Zn	-	Zinc

ABSTRACT

Background and Objectives:

Myocardial infarction is the single largest killer disease in the world. The extensive mortality and morbidity associated with coronary artery disease has stimulated the search for non invasive methods to assist the clinician in the diagnosis of myocardial infarction.

Oxidative stress and altered lipid profile are the hallmarks in patients of acute myocardial infarction as observed in recent years. The objectives of the study was to compare lipid peroxidation, antioxidant status and lipid profile in patients of acute MI and controls.

Methods:

The present study comprises of 50 clinically diagnosed and ECG confirmed cases of acute MI admitted to the intensive cardiac care unit of KLE Society's Dr Prabhakar Kore Hospital and Research Centre, Belgaum. This study was undertaken between September 2007 to August 2008.

The cases were grouped into diabetic (25) and hypertensives (25). All the patients were in the age group of 40-60 years including both the sexes. Biochemical parameters like malondialdehyde, enzymatic antioxidants : superoxide dismutase, catalase, glutathione reductase and non enzymatic antioxidants like vitamin E, vitamin C were estimated in cases and controls. Lipid profile was also measured in healthy controls and the cases. Blood glucose and blood pressure values were also taken.

Results:

The blood malondialdehyde (in nmol/ml) in controls was 6.3 ± 0.92 and 11.5 ± 0.72 in cases. The value of superoxide dismutase (IU/g of Hb) in controls was 940.4 ± 93.95 and 450.8 ± 116.76 in the cases. The mean glutathione reductase level (in IU/g of Hb) in controls was 9 ± 1.02 and 3.1 ± 0.79 in the cases. The mean catalase level (in IU/g of Hb) in controls was 7.3 ± 0.70 and 2.6 ± 0.53 in cases. The mean level of vitamin E (in mg/dl) in controls was 0.92 ± 0.10 and 0.54 ± 0.14 in the cases. The mean vitamin C level (in mg/dl) in controls was 0.87 ± 0.09 and 0.51 ± 0.13 in the case.

The mean value of total cholesterol (in mg/dl) in controls was 170.6 ± 21.38 and 260 ± 28.12 in the cases. The value of HDL (in mg/dl) in controls was 39.6 ± 4.17 and 31.1 ± 0.83 in the cases. The mean level of LDL (in mg/dl) in controls was 118.8 ± 27.43 and 151.3 ± 20.03 in the cases. The mean level of VLDL (in mg/dl) in controls was 25.5 ± 5.51 and 34.2 ± 10.54 in cases. The mean level of triglyceride (in mg/dl) in controls and cases was 118.4 ± 23.79 and 157.7 ± 20.21 respectively.

The mean level of blood sugar (in mg/dl) and B.P. (in mm of Hg) was increased in diabetic and hypertensives with acute MI.

Interpretation and conclusion:

Our study revealed that there was increased oxidative stress and decreased antioxidant defense in patients of acute myocardial infarction. Altered lipid profile was also observed in the study.

It is established fact that diabetic and hypertensive individuals are more prone for AMI as compared to healthy individuals.

Supplementation of natural antioxidants to the individuals who are prone for AMI (i.e, diabetic and hypertensive) may prevent the occurrence of AMI to a certain extent.

Key words: Acute myocardial infarction; lipid peroxidation; antioxidants; lipid profile.

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INTRODUCTION

Myocardial infarction (MI) is the single largest killer disease in the world.[1] Death from cardiovascular disease has overtaken all the other causes of mortality. It causes damage to heart muscle by blocking oxygen and nutrient supply. MI is responsible for many years of potential life lost in the middle age irrespective of gender or race.

Oxygen is vital for most organisms, but paradoxically is the source of oxygen free radical molecules which cause damage to key biological sites. Free radical reactions are ubiquitous in living things. They are produced by enzymatic and non-enzymatic reactions.[2]

Oxidation reactions ensure that molecular oxygen is completely reduced to water. The products of partial reduction of oxygen are called Reactive Oxygen Species (ROS) which are highly reactive and make havoc in the living organisms.[3]

1) Super oxide anion radical ($\bar{O}_2$)
2) Hydroperoxyl radical ($HCOO^{\bullet}$)
3) Hydrogen peroxide (H_2O_2)
4) Hydroxyl radical ($OH^{\bullet}$)
5) Lipid peroxide radical ($ROO^{\bullet}$)
6) Singlet Oxygen (O_2)
7) Nitric Oxide ($NO^{\bullet}$) and
8) Peroxy Nitrite ($ON\bar{O}\bar{O}$)

Characteristic features of Reactive Oxygen Species:

- Extreme reactivity
- Short life span
- Generation of new ROS by chain reaction
- Damage to various tissues

Damage produced by Reactive Oxygen Species:

I) Protein: resulting in loss of function

II) Lipid: peroxidation leading to membrane damage

III) Mitochondria: damage to mitochondria

IV) DNA: causing cell death, mutation and cancer.

Polyunsaturated fatty acids (PUFA) are normal constituents of cellular and sub-cellular membranes. The integrity of cell membrane is affected by peroxidation of PUFA. Lipid peroxidation of membrane proceeds as a chain reaction and it occurs in 3 steps:- i) Initiation phase ii) Propagation phase

iii) Termination phase

Many defense mechanisms exist in the body i.e. antioxidants to limit the levels of reactive oxygen species. These include –

A) Enzymatic

- Superoxide dismutase (SOD)
- Glutathione peroxidase
- Glutathione reductase
- Catalase

B) Non-enzymatic

- Vitamin A (β – carotene)
- Vitamin E (α – tocopherol)
- Vitamin C (Ascorbic acid)
- Cerulloplasmin
- Uric acid
- Bilirubin etc.

In biological system, oxidative stress refers to a disturbance in the pro-and antioxidants, balance in favor of the pro-oxidants. Oxidative stress ensues when ROS evade or overwhelm the antioxidant protective mechanisms of cells and tissues.[4]

Oxidative stress induced by reactive oxygen species is implicated in the pathogenesis of a variety of vascular diseases, including atherosclerosis, hypertension and coronary artery disease. Due to their highly reactive and non specific nature, ROS can attack almost all the biomolecules including lipid membranes.[5]

Lipid peroxidation and consequent degradation products such as malondialdehyde (MDA) (-CHO-CH_2-CHO-) are seen in biological fluids. The estimation of MDA is one of the parameters used to assess the oxidative stress.

Low Density Lipoprotien (LDL) is deposited under the endothelial cells, which undergo oxidation by free radicals released from the endothelial cells. Oxidized LDL attracts macrophages which are then converted into foam cells. Such foam cells are deposited in the endothelium leading to the formation of “fatty streaks” and finally the atherosclerotic plaque. The endothelial cell damage, smooth muscle cell proliferation, uncontrolled lipid uptake, decreased prostaglandin synthesis and associated thrombogenecity are strongly implicated in the pathogenesis of atherosclerosis.

In recent years oxidative stress and antioxidant defense play a major role in the genesis and protection of acute MI. So the present study is undertaken to assess oxidative stress by measuring malondialdehyde and antioxidant defense by estimating the levels of superoxide dismutase, glutathione reductase, catalase, vitamin E, vitamin C and blood lipid profile in patients of acute myocardial infarction.

OBJECTIVES OF THE STUDY

1. To assess oxidative stress in AMI by measuring a lipid peroxidation product MDA and antioxidant defense by measuring enzymatic antioxidants like superoxide dismutase, catalase, glutathione reductase and non-enzymatic antioxidants vitamin E, vitamin C.
2. To study lipid profile, blood-sugar and BP in the patients.
3. To compare the values of the above parameters with that of controls.

REVIEW OF LITERATURE

Acute myocardial infarction (AMI) is a worldwide epidemic.[1] 40 million people are estimated to be suffering from cardiovascular disease in the world. In India prevalence of coronary heart disease (CHD) in adults was found to be 31.9 per 1000 in men and 25.3 per 1000 in women.

Now India is in a stage of epidemiological transition, with infectious diseases gradually contributing less to morbidity and mortality and the non-communicable diseases like Ischemic Heart Disease (IHD) assuming a more menacing proportion.[6]

AMI may strike an individual during the most productive years so it can have profoundly deleterious psychosocial and economic ramifications. The early (30 – days) mortality rate from AMI is ~30%, with more than half of these deaths occurring before the patient reaches the hospital[7].

Acute Myocardial Infarction (AMI) is defined as the necrosis of heart muscle resulting from ischemia, due to an imbalance between oxygen supply and demand. AMI occurs when the coronary blood flow decreases abruptly after a thrombotic occlusion of a coronary artery.

Atherosclerosis is the general term for hardening of the arteries, characterized by the deposition of cholesterol and LDL in the arterial wall, which leads to formation of plaque and results in the endothelial damage and narrowing of the lumen[8]. Atherosclerosis affects different blood vessels and yields distinct clinical manifestations depending on the circulatory area affected. Atherosclerosis of the coronary arteries causes myocardial infarction.

The prevalence and severity of atherosclerosis and Ischemic Heart Diseases (IHD) among individuals and groups are related to several risk factors. Multiple risk

factors have a multiplicative effect; two risk factors increase the risk approximately fourfold. When three risk factors are present (e.g. hyperlipidemia, hypertension and smoking), the rate of MI is increased seven times[9].

Table No.1: Risk factors of Atherosclerosis

Major Risks	Others
1) Non modifiable ▪ Age ▪ Sex ▪ Family history ▪ Genetic factor 2) Modifiable ▪ Hyperlipidemia ▪ Hypertension ▪ Cigarette smoking ▪ Diabetes Mellitus (DM)	▪ Obesity ▪ Physical inactivity ▪ Stress ▪ Post menopause (estrogen deficiency) ▪ High carbohydrate intake ▪ Lipoprotien (a) ▪ Hardened (trans) unsaturated fat intake ▪ Chlamydia pneumonia infection ▪ Alcoholism

1) Age: Ageing brings about changes in the blood vessel wall due to decreased metabolism of cholesterol. As age advances, the elasticity of the vessel wall decreases and leads to a turbulent flow of blood and invites atherosclerosis.

2) Sex: Decades of observational studies have verified excess coronary risk in men compared with premenopausal women. Sex incidence is equal after 65 yrs of age, suggesting that male sex hormone might be atherogenic and conversely female sex hormones (estrogen) might be protective.

3) Genetic factor: Hereditary genetic derangement of lipoprotein metabolism leads to high blood lipid level and familial hypercholesterolemia predisposing to atherosclerosis.

4) Hyperlipidemia: Hyperlipidemia is the major risk factor. The following components of plasma lipids are associated with increased risk:

I) Serum Cholesterol → The value should be preferably below 200 mg/dl. Values around 220 mg/dl will have moderate risk and values above 240 mg/dl need active treatment.

II) LDL Cholesterol → Below 130 mg/dl is desirable Levels between 130 and 159 are borderline; while above 160 mg/dl carry definite risk. Hence LDL is described as "bad cholesterol". LDL transports cholesterol from liver to peripheral tissues. It has maximum association with atherosclerosis.

III) HDL- cholesterol → High Density Lipoprotein (HDL) participates in reverse cholesterol transport i.e. it transports cholesterol from cells (atherosclerotic plaques) to the liver for excretion in the bile. HDL level above 60 mg/dl protects against heart disease. As it is "anti-atherogenic" or "protective" in nature HDL is known as "good cholesterol" or Highly Desirable Lipoprotein.

The level of HDL-2 in serum is inversely related to the incidence of myocardial infarction. Values below 35 mg/dl increase the risk of Coronary Artery Disease (CAD). For every 1 mg/dl drop in HDL, the risk of heart disease rises 3%.The ratio of total cholesterol / HDL > 3.5 is dangerous. Similarly, LDL: HDL ratio > 2.5 is also deleterious.

IV) Lipoprotein (a) → Lipoprotein (a) is very strongly associated with myocardial infarction and is sometimes called the "little rascal". Levels more

than 30 mg/dl increases the risk 3 times; and when increased Lp(a) is associated with increased LDL, the risk is increased 6 times. This is an independent risk factor for the genesis of coronary artery disease at a young age.

Lipoprotein (a) has a significant homology with plasminogen. So it interferes with plasminogen activation and impairs fibrinolysis. This leads to unapposed intravascular thrombosis and possible myocardial infarction. Indians have a higher level of Lipoprotein (a) than Europeans[3].

5) Hypertension: A wealth of epidemiologic data supports the relationship between hypertension and atherosclerotic risk. Systolic blood pressure > 160 mm of Hg increases the risk of CAD. Every 6th Indian has a high blood pressure, and 50% of such individuals are prone to get a myocardial infarction. Mechanical injury of the arterial wall due to increased blood pressure is the proposed mechanism.

6) Cigarette smoking: Ten cigarettes per day increases the risk three-fold due to reduced level of HDL and accumulating carbon monoxide that may cause endothelial cell injury. Nicotine of cigarette will cause lipolysis and thereby increase the acetyl coA and cholesterol synthesis. Nicotine also causes transient constriction of the coronary arteries. Cessation of smoking decreases the risk to normal after 1 year[8].

7) Diabetes Mellitus: Ageing and rampant obesity underlie the current epidemic of type II DM. In diabetes, the hormone sensitive lipase is activated, so more free fatty acids are formed which are catabolised to produce acetyl coA. Hence acetyl coA pool is increased and a larger fraction is channeled to cholesterol

synthesis. Glycation of LDL prolongs its half life[3]. Oxidised LDL is taken up by nonspecific endocytosis further leading to atherosclerosis.

8) Homocysteine level: Elevated plasma homocysteine levels are an independent cardiovascular risk factor that correlates with the severity of CAD[10]. Plasma level of homocysteine above 15 micromol / L is known to increase the risk of coronary artery disease and stroke at a younger age. An increase of every 5 micromol / L of homocysteine in serum elevates the risk of coronary artery disease by as much as cholesterol increases of 20 mg/dl. Dietary supplementation with folate, vitamin B_{12} and vitamin B_6 leads to reduction in circulating levels of homocysteine. Homocysteine, which is thought to be toxic to the vascular endothelium, is converted into harmless compounds by the action of enzymes that require folate, vitamin B_6 and vitamin $B_{12.}$

9) Inflammation as marked by C - Reactive Protein (CRP)

CRP is one of the first acute phase proteins to become elevated in inflammatory response. Levels of CRP in plasma usually rise dramatically after MI as inflammation is present during all stages of atherosclerosis. Normal value of CRP is 0.05 – 0.2 mg/L.

10) Dysregulated coagulation or Fibrinolysis:

Thrombosis ultimately causes the gravest complications of atherosclerosis. The stability of an arterial thrombus depends on the balance between fibrinolytic factors, such as plasmin, and inhibitors of the fibrinolytic system such as plasminogen activator inhibitor – 1 (PAI – 1). Elevated levels of PAI – 1 are strong predictors of risk for major atherosclerotic events, including MI and stroke.

11) Other risk factors include:

- Lack of exercise
- Stressful lifestyle
- Obesity
- High carbohydrate intake
- Hardened (trans) unsaturated fat intake
- Alcoholism etc.

Pathogenesis

I) The oxidants like super oxide radicals, nitric oxide, H_2O_2 (hydrogen Peroxide) and others cause oxidation of blood LDL. The oxidized LDL is taken up by the macrophages of immune system. When these macrophages are engorged with lipid, they are called foam cells.

These foam cells become trapped in the walls of the blood vessels and contribute to the formation of atherosclerotic plaques that cause arterial narrowing and leads to heart attack.

II) The plaque is composed of smooth muscle cells, connective tissue, lipids and debris that accumulate in the intima of the arterial wall. The plaque progresses with age in the following sequence.

- The endothelial cells of the arterial wall are injured either by oxidized LDL or mechanically.
- The injured area is exposed to blood and attracts monocytes which are converted to macrophages that engulf oxidized LDL and converted to

foam cells, which accumulate causing a fatty streak to develop within the blood vessel.

III) Damaged endothelial cells cannot produce prostaglandin I_2 (PGI_2) and prostacyclin (which inhibits platelet aggregation) and hence platelets begin to aggregate and release thromboxane A_2 (TXA_2). TXA_2 stimulates platelet aggregation.

IV) Damaged endothelial cells also release platelet – derived growth factor (PDGF) that causes proliferation of smooth muscle cells, which contribute to the formation of atherosclerotic plaque that cause arterial narrowing and leads to heart attack [8,9,10]

Atheromatous plaques are susceptible to the following pathologic changes –

- Haemorrhage
- Rupture, ulceration or erosion
- Atheroembolism
- Aneurysm formation
- Calcification

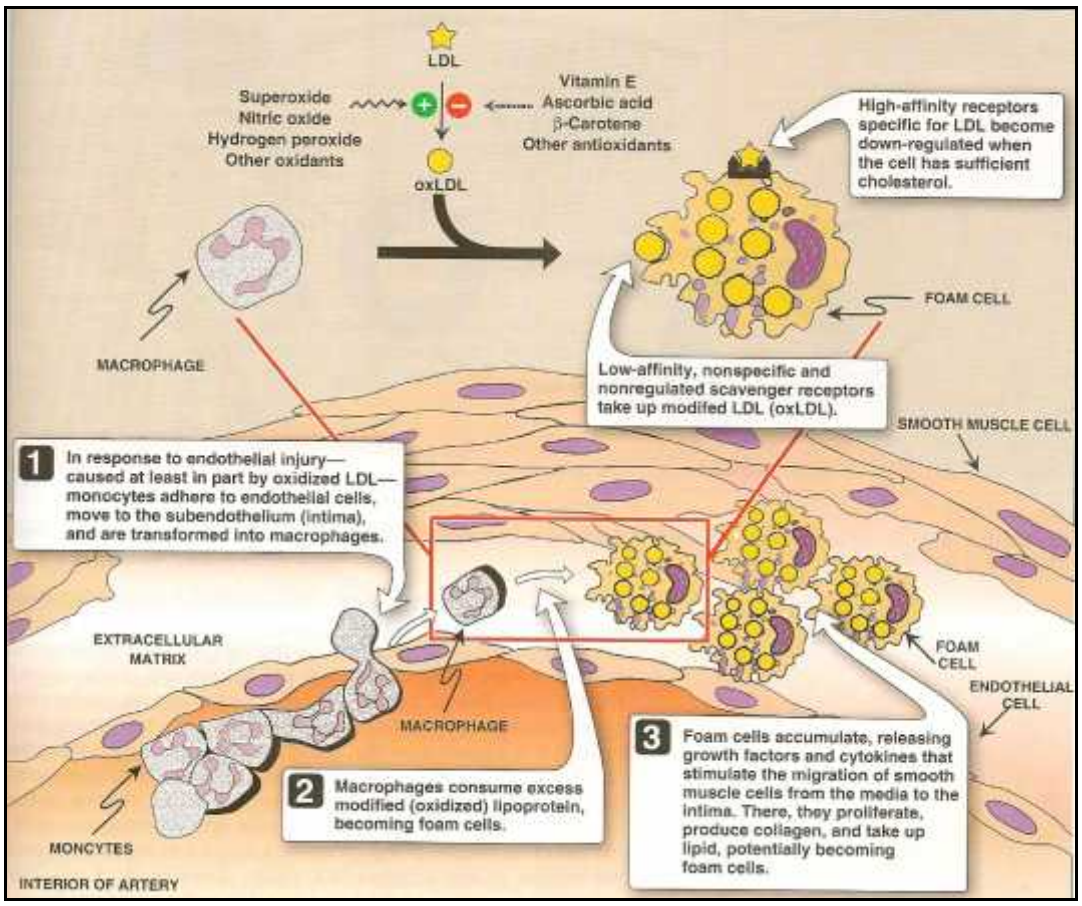


Fig. 1: Role of oxidized lipoproteins in plaque formation in arterial wall [10]

Prevention of atherosclerosis

a) Dietary restriction

The most important preventive measure against the development of atherosclerosis is to eat a low fat diet that contains mainly unsaturated fat with low cholesterol content. The accepted standard of fat intake is 20% of total calories. The recommended daily allowance (RDA) is about 20 – 25g of oils and 2 – 3g of polyunsaturated fatty acid (PUFA).

Sunflower oil and fish oils contain PUFA. They are required for the esterification and final excretion of cholesterol. So PUFA is helpful to reduce cholesterol level in the blood. Omega – 3 fatty acids from fish oils reduce LDL and decrease the risk of MI.

HDL is associated with a protein, (Apo A_1) that has esterase activity which degrades and destroys the oxidized LDL, accounting for HDL's ability to protect against heart disease[3,8]. LDL contains Apo B protein which is involved in rapid progression of atherosclerosis.

Sucrose has a significant effect in raising plasma triglycerides. High carbohydrate diet should be avoided. Intake of green leafy vegetables is recommended. Due to their high fibre content, they increase the motility of bowels and reduce reabsorption of bile salts thus lower cholesterol levels[3].

b) Moderate consumption of alcohol and exercise appears to have a beneficial effect by raising the level of HDL.

c) Control of diabetes and hypertension

d) Avoidance of cigarette smoking

e) Drug therapy: Lovastatin, clofibrate and cholestyramine are known to reduce cholesterol levels.

f) Administration of anti-oxidants like vitamin E, vitamin C as a supportive treatment in hyperlipidemia is now gaining significance.[3,8]

Clinical Presentation

Symptoms:-

Chest pain is the common presenting complaint involving the epigastrium and often radiating to left arm, abdomen, back, lower jaw and neck.

- Nausea, vomiting.
- Weakness
- Anxiety
- Breathlessness
- Collapse / syncope

Physical findings - Patient is anxious and restless, presence of pallor

Systemic findings - Precordium is silent

Apical pulse is difficult to palpate

Decreased intensity of first heart sound

Paradoxical splitting of second heart sound

Appearance of third and fourth heart sounds

A transient mid systolic or late systolic murmer heard

Laboratory findings:

The laboratory tests of value in confirming the diagnosis may be divided into-

1) Electrocardiogram (ECG)
2) Serum cardiac biomarkers
3) Cardiac imaging
4) Lipid profile assisted by oxidative stress (oxidant stress / antioxidant defense)

1) ECG finding: ST segment elevation
   T wave inversion and
   Q wave formation

2) Serum Cardiac Biomarkers:

Cardiac troponins (CT-I and CT-T) → Troponin I is released into the blood within 4 hours after the onset of cardiac symptoms, peaks at 14-24 hrs and remains elevated for 3-5 days post-infarction.

CI-I > 1.5 mg/L is indicative of myocardial damage.

Serum level of Troponin T increases within 6 hours and then remains elevated up to 7-10 days. The T_nT_2 estimation is 100% sensitive index for myocardial infarction[3].

Creatine phosphokinase (CK) → The CK level starts to rise within 3-6 hours of infarction, reaches peak by 12 hours and returns to normal by 3-4 days. Normal serum value is 15-100 U/L for males and 10-80 U/L for females.

Lactate Dehydrogenase (LDH) → The isoenzymes of LDH (LDH_1 and LDH_2) increase in serum within 24-48 hours after an infarct and remain elevated for 7-10 days. Normal value of LDH in serum is 100-200 U/L.

Aspartate aminotransferase (AST) → Although AST increases following MI, its use in the diagnosis of myocardial infarction is limited by its nonspecificity. Normal serum value of AST is 8-20 U/L.

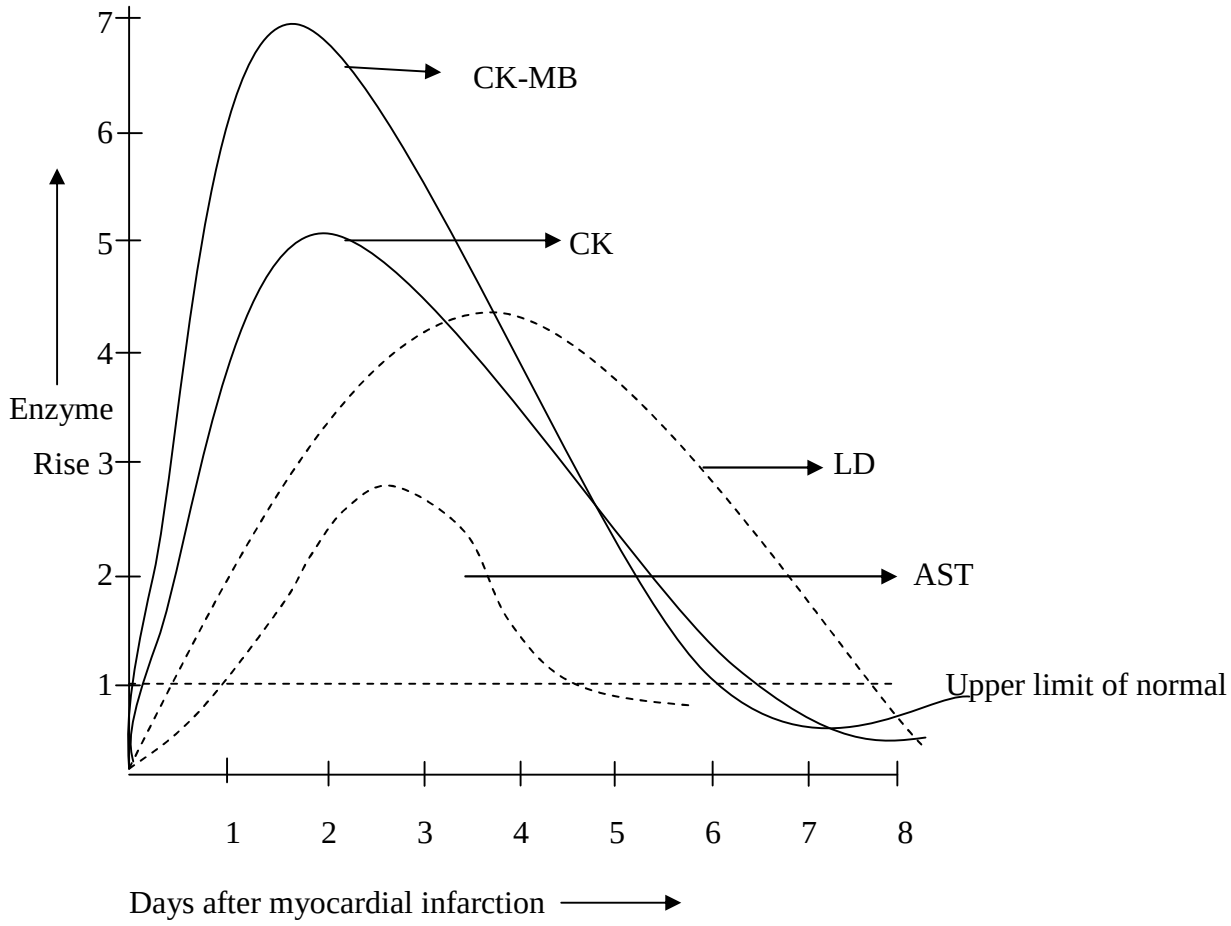


Fig No. 2. Serum enzyme activities following myocardial infarction[11].

3) Cardiac imaging

Echocardiographic estimation of left ventricular function (LV) is useful prognostically. It may also identify the presence of right ventricular (RV) infarction, ventricular aneurysm, pericardial effusion and LV thrombus.

4) Lipid profile: Elevation of serum total cholesterol

Increase in LDL and VLDL

Elevated levels of triglycerides

Decrease in the level of HDL.

Oxidative stress (oxidant stress / antioxidant defense)

- Increased level of malondialdehyde
- Decreased level of antioxidant enzymes like Superoxide dismutase (SOD), catalase, glutathione reductase etc

Complications of myocardial infarction

- Ventricular dysfunction
- Congestive heart failure
- Cardiogenic shock
- Right ventricular infarction
- Arrythmias – Ventricular premature beats

  Ventricular tachycardia and fibrillation

  Sinus bradycardia

  Supraventricular arrhythmias

- Other complications like

  Recurrent chest discomfort

  Pericarditis

  Thromboembolism

  Left ventricular aneurysm[12]

FREE RADICALS

A free radical is any species capable of independent existence that contains one or more unpaired electrons. An unpaired electron is one that is alone in an orbital.[13] The unpaired electron gives certain characteristic properties to the free radical, such as para magnetism. The chemical reactivity of free radicals is usually high. They may be positively charged, negatively charged or electrically neutral.[14] A free radical is conventionally represented by a superscript dot ($R^{\bullet}$).

A compound becomes a free radical by gaining an additional electron, as in the case of reduction of molecular oxygen to superoxide anion radical ($O_2^{\bullet -}$). Other free radicals are hydroperoxyl radical ($HOO^{\bullet}$), hydroxyl radical ($OH^{\bullet}$), lipid peroxyl radical ($ROO^{\bullet}$). The sequential univalent reduction steps of oxygen may be represented as,[15]

$$O_2 \xrightarrow{(+)\ e^-} O_2^{\bullet -} \xrightarrow{(+)\ e^{-},\ 2H} H_2O_2 \xrightarrow[-H_2O]{(+)\ e^{-},\ H^+} OH^{\bullet} \xrightarrow{(+)\ e^{-},\ H^+} H_2O$$

Generation of Free Radicals:

Free radicals may be formed by;

a. Cleavage of a covalent bond of a normal molecule.

b. Loss of a single electron from a normal molecule and

c. Addition of single electron to a normal molecule.

Sources of Free Radicals:

Oxidants related to human disease are derived from three sources.

- Those generated via normal intracellular biologic processes but in an exaggerated, inappropriate fashion or in a milieu where the normal defenses that serve to protect tissues are inadequate.
- Those released by inflammatory cells into their local environment.

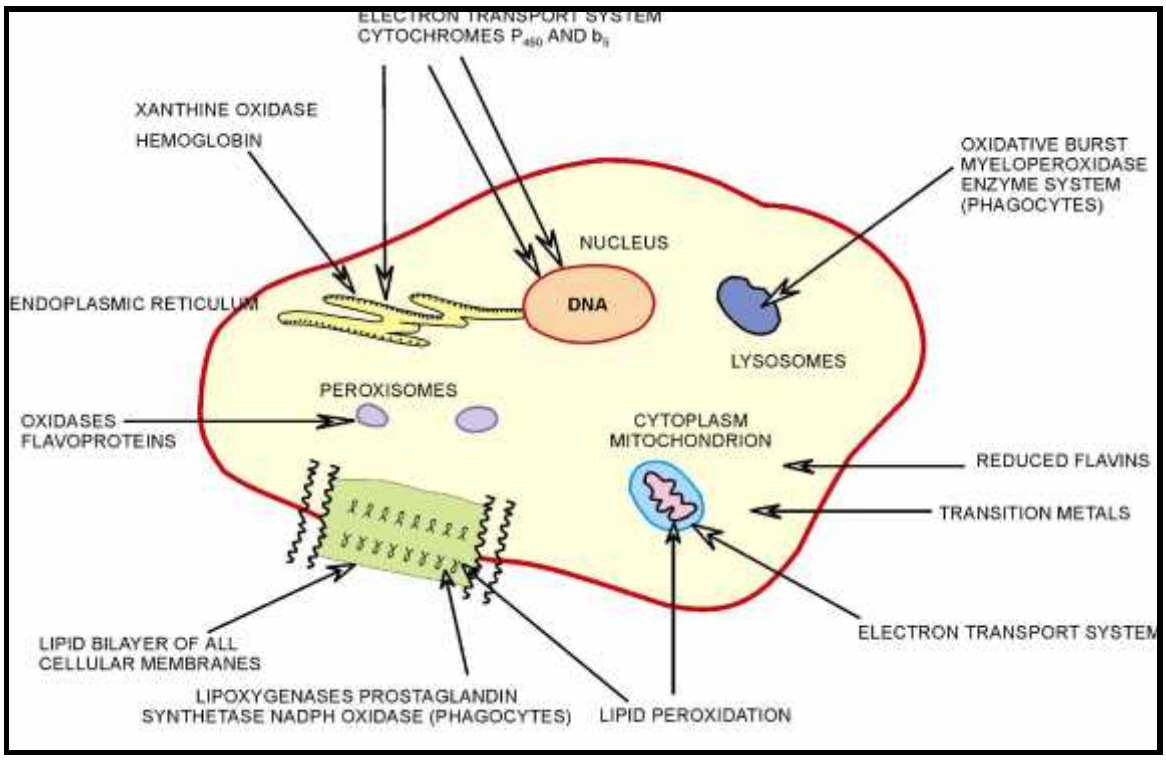


Fig. No.3. The Cellular Sources of free Radicals

Those secondary to xenobiotics, either because the xenobiotics includes oxidants (e.g. inhaled oxidant gases) or because these xenobiotics induce oxidant generation within cells, e.g. drugs that injure tissues through oxidant mechanisms.[16]

i) Endogenous Sources:

a) Endogenous Sources of free radicals include those that are generated and act intracellularly as well as those that are formed within the cells and are released into the surrounding area[17] (Fig No.3). Oxidation-reduction reactions generate free radicals constantly within the body. These can be mediated by the action of enzymes or non-enzymatically, often through the redox chemistry of transition metal ions[18].

Oxidases and electron transport systems are prime, continuous sources of intracellular reactive oxygen free metals. Electron transfer from transition metals such as iron to oxygen can initiate free radical reactions[17]. An important source of superoxide anion radical is the "univalent leak" of superoxide anion radical from the mitochondrial electron transport system[19].

b) Intracellular free radicals are generated from the auto oxidation and consequent inactivation of small molecules such as reduced flavins and thiols, and from the activity of certain oxidases, cycloxygenases, lipoxygenases, dehydrogenases and peroxidases[17] (Fig No.3). A variety of enzyme systems catalyze the univalent reduction of molecular oxygen to superoxide anion radical. Such univalent reduction of molecular oxygen also occurs invivo in non-enzymatic electron-transfer oxidation-reduction reactions. (E.g. hydroquinone + $O_2 \longrightarrow$ semiquinone + $O_2^{\bullet -}$ + H^+) and during auto-oxidation

reactions including those that involve catecholamines, flavins and reduced ferridoxins[20].

c) Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in the inflammatory cells (neutrophils, eosinophils, monocytes and macrophages) produces superoxide anion by the process of respiratory burst during phagocytosis. The superoxide is converted to hydrogen peroxide and then to hypochlorous acid (HOCI) with the help of superoxide dismutase (SOD) and Myeloperoxidase (MPO). The superoxide and hypochlorous ions are the final effectors of bactericidal action. The gene for myeloperoxidase is on chromosome 17. The enzyme myeloperoxidase has a molecular weight of 156 Kilo Daltons (KD); and contains two iron atoms per molecule. This is deliberate production of free radicals by the body. About 10% of the oxygen uptake by macrophage is used for free radical generation. Along with the activation of macrophages, the consumption of oxygen by the cell is increased drastically; this is called respiratory burst[21] (Fig No.4).

d) Hydroxyl radicals are formed in the "Fenton reaction" whenever hydrogen peroxide comes into contact with ferrous or cupric ions. An iron catalyzed Haber-weiss type of reaction may also form this radical. The net effect of which is an interaction between hydrogen peroxide and superoxide anion radical in the presence of traces of transition metal ions to form hydroxyl radical. Finally, the $OH^{\bullet}$ radical is also a product of ionizing radiation[20, 22].

$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$ (Fenton reaction)

$O2^{\bullet -} + H_2O_2 \xrightarrow{\text{Fe salt catalyst}} O_2 + OH^{\bullet} + OH^{-}$.

(Haber Weiss reaction)

The chelated Fe^{3+} can be reduced to Fe^{2+} by thiols, ascorbate and most of the other reductants. Fe^{2+} can then auto-oxidize, producing $O_2^{\bullet -}$

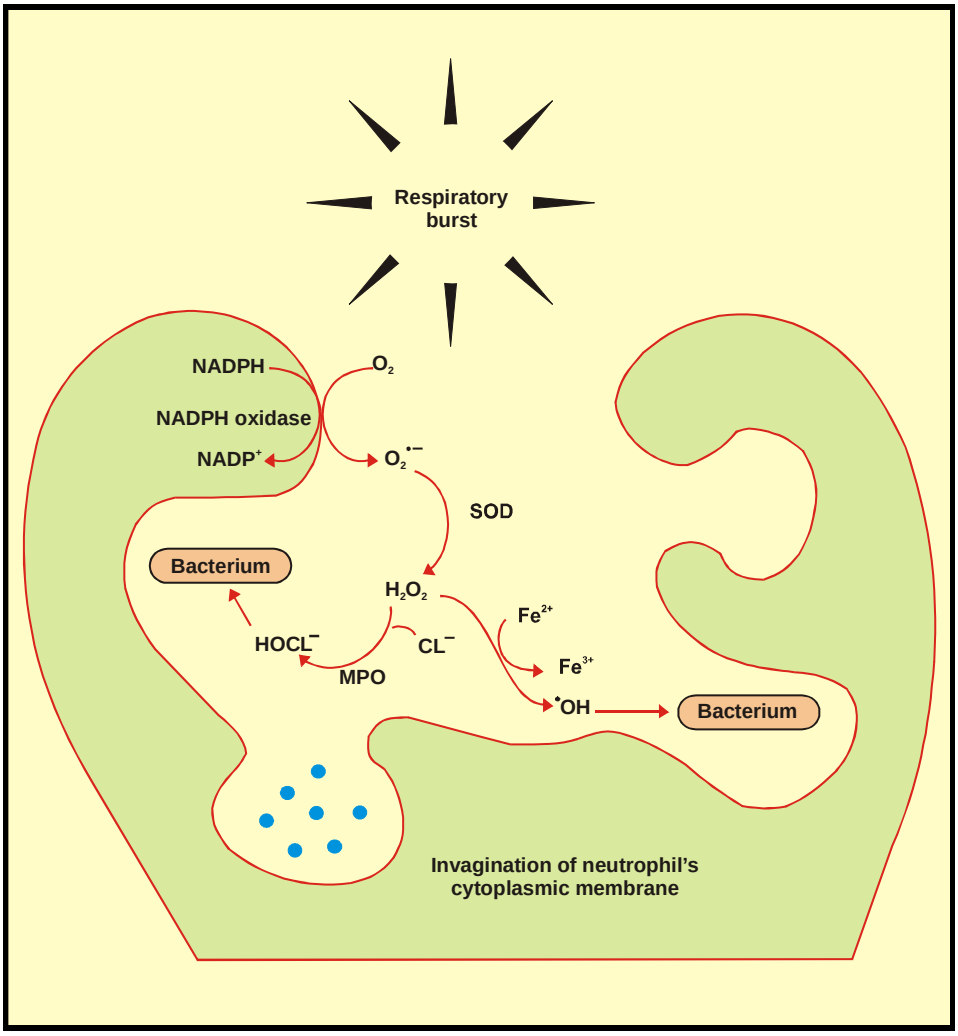


Fig. No. 4. Generation of Oxygen Free Radicals in Respiratory Burst

ii) Exogenous Sources:

a) Cigarette smoke contains high concentrations of various free radicals. Other toxic compounds such as carbon tetrachloride, drugs and inhalation of air pollutants, anaesthetics, organic solvents, hypertoxic environments and pesticides will increase the production of free radicals[17].

b) Ionising radiation damages tissues by producing hemolytic fission of background water.

$H_2O \longrightarrow$ (gamma, UV radiation) $\longrightarrow H^{\bullet} + OH^{\bullet}$

c) Light of appropriate wavelengths can cause excitation of oxygen in presence of photosensitizers to produce singlet oxygen.

Sites of Free Radical Generation:

Main sites of free radical generation are mitochondria, lysosomes, peroxisomes, nuclei, endoplasmic reticulum, plasma membranes and the cytosol[17].

i) Endoplasmic Reticulum, Nuclear Membrane and Electron Transport Systems:

Free radicals produced by the endoplasmic reticulum and nuclear membrane can undergo both intraorganelle and cytosolic reactions. In case of nuclear membrane generated radicals DNA would be particularly susceptible to free radical damage.

ii***) Plasma Membrane:***

Plasma membrane is a site of action of extracellularly generated free radicals. They must cross the plasma membrane before reacting with other cell components and may initiate toxic reactions at the membrane. The unsaturated fatty acids present in membrane and transmembrane proteins containing oxidizable aminoacids are susceptible to free radical damage. Increased membrane permeability caused by lipid peroxidation or oxidation of structurally important proteins can cause breakdown of transmembrane ion gradients, resulting in loss of secondary functions and inhibition of integrated cellular metabolic processes.

The interior of biological membranes is hydrophobic and $O_2^{\bullet -}$ produced in the environment could be extremely damaging. Much of the $O_2^{\bullet -}$ generated within cells comes from membrane bound systems and it is certainly possible that some of it is formed in the membrane interior[23].

iii) Peroxisomes:

Peroxisomes are potent sources of cellular hydrogen peroxide because of high concentrations of oxidases.

REACTIVE OXYGEN SPECIES (ROS)

The oxygen derived free radicals and related non-radical species are collectively known as reaction oxygen species (ROS). Several reactive oxygen species (ROS) are known, among them the most prominent are:

Superoxide Radical ($O_2^{\bullet -}$):

This ROS is formed, when oxygen takes up one electron and as leaks in the mitochondrial electron transport but its formation is easily increased when exogenous components (redox cycling compounds) are present. Its first production site is the internal mitochondrial membrane (NADH ubiquinone reductase and ubiquinone cytochrome-c-reductase).[24] This species is reduced and forms hydrogen peroxide (H_2O_2). The production of superoxide radicals at the membrane level (NADPH oxidase) is initiated in specialized cells with phagocytic functions (macrophages) and contributes to their bactericidal action (oxidative burst) [25]. The flavin cytosolic enzyme xanthine oxidase found in quite all tissues and in milk fat globules generates superoxide radicals from hypoxanthine and oxygen and is supposed to be at the origin of vascular pathologies.

Hydrogen Peroxide (H_2O_2):

Hydrogen peroxide is mainly produced by enzymatic reactions. These enzymes are located in microsomes, peroxisomes and mitochondria. Even in normal conditions, the hydrogen peroxide production is relatively important and leads to a constant cellular concentration between 10^{-9} and 10^{-7} M. In plant and animal cells, superoxide dismutase is able to produce H_2O_2 by dismutation of $O_2^{\bullet -}$, thus contributing to the lowering of oxidative reactions. The natural combination of dismutase and catalase contributes to remove H_2O_2 and thus has a true cellular antioxidant activity. H_2O_2 is also able to diffuse easily through membranes.[26]

Hydroxy Radical ($OH^{\bullet}$):

In the presence of Fe^{2+}, H_2O_2 produces the very active species $OH^{\bullet}$ by the Fenton reaction.

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

The iron-catalyzed decomposition of oxygen peroxide is considered the most prevalent reaction in biological systems and the source of various deleterious lipid peroxidation products.

Nitric Oxide ($NO^{\bullet}$):

Nitric Oxide is produced in vascular endothelium. This species is not too reactive (poorly oxidizing function), it reacts readily with $O_2^{\bullet -}$ and gives the extremely reactive peroxynitrite ($ONOO^{-}$). This ROS is naturally formed in activated macrophages[27] and endothelial cells[28] and is considered as an active agent in several pathologies based on inflammation, organ reperfusion and also may play an important role in atherosclerosis.

Single Oxygen (O_2):

This chemical form of oxygen is not a true radical but is reported to be an important ROS in reactions related to ultraviolet exposition (UVA 320-400 nm). Its toxicity is reinforced when appropriate photoexcitable compounds (sensitizers) are present with molecular oxygen[29]. Several natural sensitizers are known to catalyze oxidative reactions such as tetrapyrroles (bilirubin), flavins, chlorophyll, hemoproteins and reduced pyridine nucleotides (NADH). Some of these sensitizers are also found in foods and cosmetics. Some others are used for therapeutic purposes (anti-cancer treatments) and are sensitive to visible light. The presence of metals contributes to increase in the production of singlet oxygen, as well as anion superoxide, and thus accelerates the oxidation of unsaturated lipids generating hydroperoxides. It has been suggested that singlet oxygen may be formed during the degradation of lipid peroxides and thus may cause the production of other peroxide molecules. This singlet oxygen formation may account for the chemiluminescence observed during lipid peroxidation.[30]

Thiyl Radicals ($RS^{\bullet}$):

Thiol compounds (RSH) are frequently oxidized in the presence of iron or copper ions.

$$RSH + Cu^{2+} \longrightarrow RS^{\bullet} + Cu^{+} + H^{+}$$

These thiyl radicals have strong reactivity in combining with oxygen.[31, 32]

$$RS^{\bullet} + O_2 \longrightarrow RSO^{\bullet}_2$$

Furthermore, they are able to oxidize NADH into NAD, ascorbic acid and to generate various free radicals ($^{\bullet}OH$ and $O2^{\bullet-}$). These thiyl radicals may also be formed by hemolytic fission of disulfide bonds in proteins.

Carbon Centered Radicals:

The formation of this reactive free radical is observed in cells treated with carbon tetrachloride (CCl_4). The action of the cytochrome P_{450} systems generates the trichloromethy1 radical ($^{\bullet}CCl_3$), which is able to react with oxygen to give several peroxy1 radicals (i.e. $^{\bullet}O_2\,CCl_3$).[33]

DAMAGE PRODUCED BY FREE RADICALS

Free radicals are extremely reactive. Their mean effective radius of action is only $30A^0$. Their half-life is only a few milliseconds. When a free radical reacts with a normal compound, other free radicals are generated. This chain reaction leads to thousands of events. The peroxidation of polyunsaturated fatty acids (PUFA) severely damages the cell membrane leading to loss of membrane functions like absorption, secretion etc. Almost all biological macromolecules are damaged by the free radicals e.g.

a) peroxidation of PUFA in plasma membranes,

b) Oxidative inactivation of sulfhydry1 containing enzymes,

c) Polysaccharide depolymerization and DNA breaks.

d) DNA damage may directly cause inhibition of protein and enzyme synthesis, indirectly it also cause cell death or mutation and carcinogenesis.

e) Lipid peroxidation and consequent degradation product such as MDA seen in biological fluid. Their effect in the serum is often employed to assess the oxidant stress[34].

Proteins:

Protein molecules undergo substantial modifications through reactive reactions with free radicals. Proteins containing tryptophan, tyrosine, phenylalnine histidine, methionine and cysteine can undergo free radical mediated amino acid modification. Free radical promote sulfhydryl mediated cross-linking of such labile amino acids as well as cause fragmentation of polypeptide chains. Oxidative modifications enhance degradation of critical enzymes by cytosolic neutral proteases[35]. Enzymes undergo cross-linking with resulting increase in molecular weight, such enzymes cross-link with their neighbours in a random destructive reaction. The normal precision arrangement of protein and enzymes in subcellular membranes and organelles is badly disrupted and their biological properties are lost or impaired.[36]

Carbohydrates:

Advances in free radical chemistry indicate that no biological substance is impervious to free radical attack. Therefore, it is not surprising that glucose and other related monosaccharides undergo oxidation but conditions are appropriate.[35] Hyaluronic acid undergoes polymer fragmentation following exposure to free radical system, which leads to destabilization of connective tissue and loss of synovial fluid viscosity.

Nucleic Acid:

DNA is readily attacked by oxidizing radicals if they are formed in its vicinity has been clearly demonstrated by radiation biologists. It must therefore be considered as a vulnerable and important target. Cell mutation and death from ionizing radiation is primarily due to free radical reactions with DNA. Cell death and mutations arising

from free radicals generated during normal metabolism have also been ascribed to reactions with DNA[37].

Lipids:

All of the major classes of biomolecule may be attacked by free radicals but lipids are probably the most susceptible[2]. Cell membranes are rich sources of polyunsaturated fatty acids. Biomembrane and organelles are the major sites of lipid peroxidation damage. Major constituents of biological membranes are lipids and proteins. Lipid peroxidation can damage membrane proteins as well as lipids.[23] The membrane fluidity is due to the presence of polyunsaturated fatty acid side chain in many membrane lipids, which lower the melting point of the interior membrane. Lipid peroxidation decreases membrane fluidity. Conditions, which favour lipid peroxidation are:

i) A high degree of unsaturation in the lipid substrate.

ii) A rich supply of oxygen and

iii) The presence of transitional metal catalysts.[38]

LIPID PEROXIDATION

Lipid peroxidation is defined as "oxidative deterioration of polyunsaturated lipids". Lipid peroxidation is particularly damaging because it proceeds as a self perpetuating chain reaction.[39]

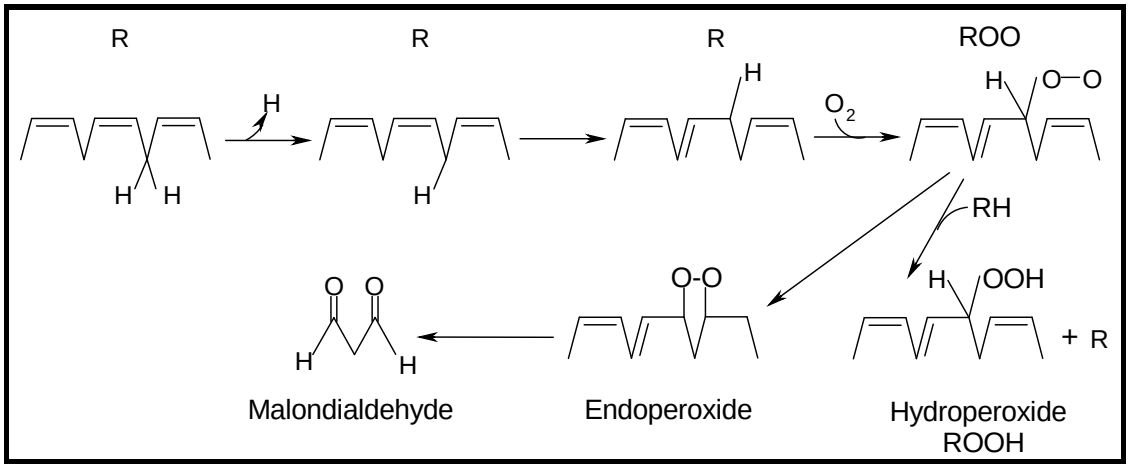


Fig. No.5: The lipid peroxidation process

Figure 5 Illustrate lipid peroxidation. The reaction is initiated by an existing free radical ($X^{\bullet}$), by light, or by metal ions. Malondialdehyde is only formed by fatty acids with three or more double bonds, and is used as a measure of lipid peroxidation together with ethane from the terminal two carbon of ω3 fatty acids and pentane from the terminal five carbon of ω6 fatty acids.[40]

Peroxidation of polyunsaturated fatty acids usually involves three operationally defined processes.[23]

Initiation Phase:

During this phase, the primary event is the abstraction of hydrogen atom from bisallylic site of PUFA. Initiation of a peroxidation sequence in membrane or PUFA[35] is due to the attack of any species that has sufficient reactivity to abstract a hydrogen atom from a methylene (CH_2) group. This leaves behind an unpaired electron on the carbon, -CH-. The carbon radical tends to be stabilized by a molecular rearrangement to produce a conjugated diene, which then easily reacts with an oxygen molecule to give peroxy radical, $R\text{-}OO^{\bullet}$. The presence of the redox active metals such as iron or copper can facilitate the initiation process.[23]

$ROOH + Metal\ (n)^{+} \longrightarrow ROO^{\bullet} + Metal^{(n-1)+} + H^{+}$

$X^{\bullet} + RH \longrightarrow R^{\bullet} + XH$

Propagation Phase:

During this phase lipid peroxidation relies on the interaction of molecular oxygen with carbon-centered free radicals to form lipid hydroperoxides.[35] The peroxy radical abstract a hydrogen atom from another lipid molecule and once the process begins it tends to continue. The peroxy radical combines with the hydrogen atom that it abstracts to give a lipid hydroperoxides R-OOH. A probable alternative fate of peroxy radicals is to form cyclic peroxides. With the help of metal catalysts, the decomposition of hydroperoxides result in the formation of alkoxyl or peroxyl radicals. These radicals are capable of further reactions and thus the propagation of lipid peroxidation continues.[23, 35]

$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$

$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$ etc

Termination Phase:

The propagation reactions of lipid peroxidation will not proceed very far before they meet a protein molecule, which can then be attacked and damaged, in addition aldehyde can attack amino groups on the protein molecule to form both intramolecular cross links and also cross links between different protein molecules. eg. malondialdehyde. Any kind of lipid free radical can react with a lipid peroxyl radical to give non-initiating and non-propagating species.

$ROO^{\bullet} + ROO^{\bullet} \longrightarrow RO\text{-}OR + O_2$

$ROO^{\bullet} + R \longrightarrow ROOR$

$R^{\bullet} + R^{\bullet} \longrightarrow RR$

TOXIC EFFECTS OF LIPID PEROXIDATION

The uncontrolled peroxidation of bio-membranes can lead to profound effects on membrane structure and function and may be sufficient to cause cell death. The toxic products generated during lipid peroxidation may be involved in damage to specific protein and transport systems critical to cell function.[41] Malondialdehyde produced by lipid peroxidation can cause cross linking and polymerization of membrane components. This can alter the intrinsic membrane properties such as ion transport, enzyme activity. Because malondialdehyde is diffusible, it will also react with nitrogenous bases of DNA[42] lipid hydroperoxides can directly inhibit enzymes.[41]

ANTIOXIDANT DEFENSE SYSTEMS

Detoxification of reactive oxygen species is one of the prerequisite of aerobic life. Many defense systems have evolved by providing an important antioxidant defense system of prevention, interception and repair consisting of non-enzymatic and enzymatic scavengers and quenchers (Fig. No. 5).[43]

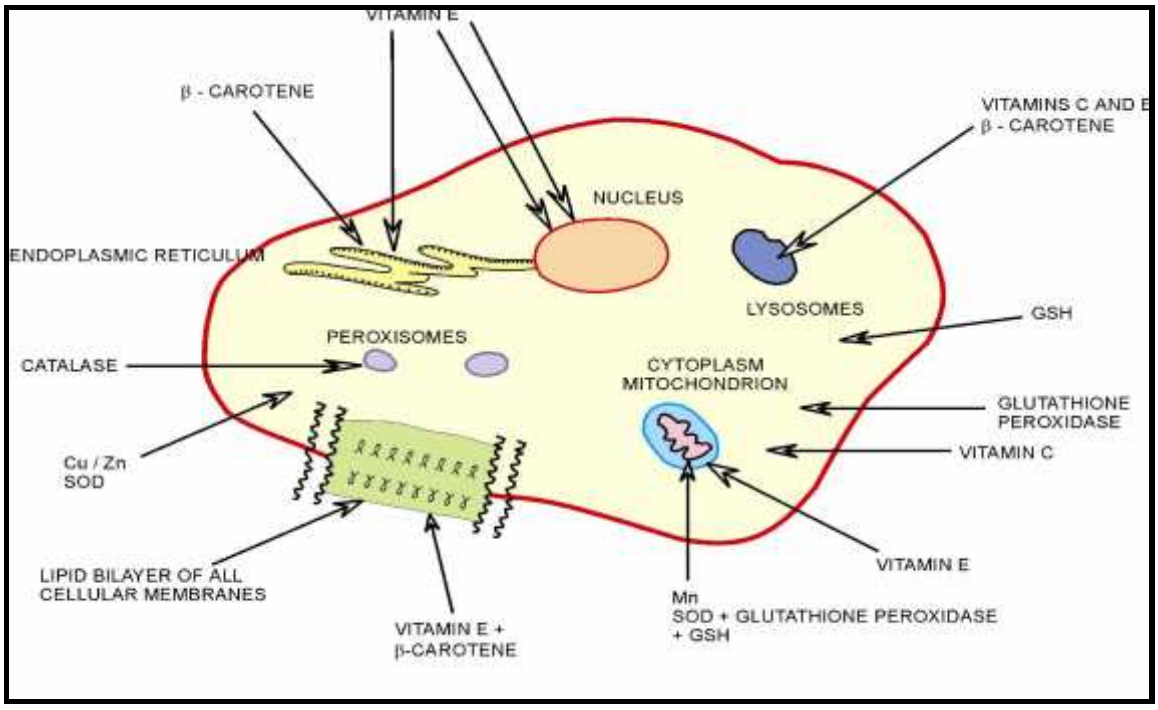


Fig No.6. Antioxidant protection within the cell

Prevention:

The important function of these antioxidant defenses is to prevent the generation of free radicals. Antioxidants defense remove peroxides that are reacting with transition metal ions to produce reactive free radicals. These peroxides include both hydrogen peroxide and also the lipid hydroperoxides, which are produced during lipid peroxidation. Catalase and glutathione peroxidases are the enzymes, their role is to safely decompose peroxides.[23] Transferrin and iron binding protein lactoferrin also function as preventive antioxidant by binding iron and stopping it from participating in radical reactions. Uric acid, albumin, haptoglobin and hemopexin have also been shown to inhibit various free radical reactions.[44,45] Cerulloplasmin an important extracellular antioxidant [46] which oxidizes Fe^{2+} to Fe^{3+} that swiftly binds to transferrin and any iron mobilized from serum ferritin.[47]

Interception:

The free radical scavenging enzyme is superoxide dismutase whose substrate is a free radical; other scavengers include lipid soluble vitamin E, vitamin A, ubiquinol and the aqueous phase compounds such as free radical scavengers like ascorbic acid glutathione, uric acid etc.[18]

Repair:

Another category of natural antioxidant defense is repair processes, which remove damaged biomolecule before their presence, which alter cell metabolism or viability. Specific enzymes repair oxidatively damaged nucleic acids. Oxidized proteins are removed by proteolytic systems and oxidized membrane lipids acted upon by lipases, peroxidases and acyl transferases.

Antioxidants oppose the toxic effect of lipid peroxides and oxygen radicals, and they limit the amount of lipid peroxides that are formed[48]. To counteract the free radical damage, the tissues have an effective antioxidant defense system. Under normal circumstances, the defense system is able to cope up with a free radical in the tissues by the antioxidant donating an electron to stabilize the free radical. In doing so it can harmlessly decay itself, or later regenerate by other antioxidants.

Kirnskey defined antioxidants are "compounds that protect biologic systems against the potentially harmful effects of processes or reactions that can cause excessive oxidation".[49] With this definition we can describe various types of biologic antioxidants, their locations within and outside of cells and mechanism of actions.

Antioxidants can be classified depending on the mechanism of action as the preventive or the chain breaking. In the former category are metal chelators and enzymes like superoxide dismutase, catalase and glutathione peroxidase. In the second category is the chain breaking agents such as alpha tocopherol, ubiquinone, beta-carotene, bilirubin and water-soluble substances such as ascorbate, GSH and urate. The defense of living eukaryotic cells against the damage caused by the activated oxygen is a complex process which involves a interrelated protective agencies for activated oxygen targets like DNA, proteins and polyunsaturated fatty acids. Peroxidation of polyunsaturated fatty acids will result in a disruption of membrane. So, the antioxidants lie functionally at the heart of this protective mechanism.

Intracellular defense system is largely dependent on the antioxidant enzymes such as GSH-Px, SOD, and catalase requiring the antioxidant micronutrients like selenium, copper, zinc and iron. Riboflavin functions as an antioxidant cofactor (FAD) needed for the glutathione reductase.

ENZYMATIC ANTIOXIDANTS

SUPEROXIDE DISMUTASE

McCord and Fridorich described the metalloenzyme superoxide dismutase.[50] Superoxide dismutase is the major intracellular antioxidant enzyme, which is essential for the survival of aerobic cells. It catalytically scavenges the superoxide radical, which appears to be important agent for toxicity of oxygen and thus provides a defense against oxygen toxicity.

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \longrightarrow H_2O_2 + O_2$$

Superoxide dismutase catalyzes the dismutation of the superoxide anion free radical ($O_2^{\bullet -}$) to hydrogen peroxide and molecular oxygen at a rate 104 times faster than spontaneous dismutation at physiological pH[51] resulting in no superoxide anion available to react with hydrogen peroxide to from hydroxy1 radical through the iron catalyzed reactions. Superoxide dismutase enzyme exists in several forms and is present in mitochondrial matrix, the cytoplasm and the extracellular fluid.

Superoxide dismutase is broadly classified into distinct classes depending on the metal ion content.

1) Cu and Zn containing SOD found in cytosol.

2) Mn containing SOD found in mitochondria.

1) Cu/Zn containing superoxide dismutase: Superoxide dismutase is present in cytosol of eukaryotic cells having a molecular weight of 32,000, and is made up of two identical subunits one Cu^{2+} and one Zn^{2+} per subunit. In the Cu/Zu SOD copper is catalytically active which oxidizes cupric to the cuprous state, while Zn appears, primarily to play a structural role.[52]

2) Manganese containing superoxide dismutase: Manganese superoxide dismutase has been isolated from human liver mitochondria.[52] It contains four subunits and has a molecular weight of 80,000.

GLUTATHIONE REDUCTASE

Glutathione reductase is present in the liver, kidney, pancreas, heart, thyroid and erythrocytes. It has a molecular weight of 44000. It catalyzes the reduction of oxidized glutathione (GSSG) to glutathione (GSH) in the presence of reduced coenzyme II ($NADPH_2$)

Glutathione reductase is NADPH dependent and in erythrocytes the main source of NADPH2 is from Hexose monophosphate shunt activity.[53] The ratios of GSH/GSSG in normal cells are kept high so there must be a mechanism for reducing GSSG back to GSH, which is achieved by glutathione reductase.

Glutathione reductase contains the FAD at its active site. NADPH reduces the FAD, which then passes its electrons on to a disulfide bridge (S-S) between two cysteine residues in the protein. The two-sulphydryl groups so formed then interact with GSSG and reduce it to 2GSH, reforming the protein disulfides.[23] Glutathione reductase is present in two forms.

1. An active form associated with FAD
2. An inactive form not bound to FAD.

Reduction of GSSG to GSH mediated by glutathione reductase whose substrate is NADPH and Glucose -6-Phosphate dehydrogenase as a source of NADPH guarantees the reduction of GSSG and maintenance of constant pool of GSH.

CATALASE:

Catalase is major antioxidant defense component present in all mammalian cell types, reacts rapidly with hydrogen peroxide inside of cells and converts it into water and oxygen.[54] Selenium dependent glutathione peroxidase also inactivates hydrogen peroxide.[55] Catalase catalyzes the breakdown of toxic hydrogen peroxide directly to water and preventing the secondary generation of toxic intermediates such as hydroxy1 radical. [56]

$$2H_2O_2 \longrightarrow O_2+H_2O$$

Catalase activity is present in almost all aerobic cells. The level of activity varies not only between tissues but also within the cell itself. High levels of catalase activity are found in liver, kidney, red blood cells, microsomes and also in cytosol.[57] Purified catalase have been shown to consist of four protein subunits, each of which contains Fe^{+3}, protoporphyrin group which is bound to its active site.[58]

Mechanism of catalase reaction:-

$$\text{Catalase } Fe^{3+} + H_2O_2 \longrightarrow \text{Compound 1}$$

$$\text{Compound 1} + H_2O_2 \xrightarrow{\text{Catalase}} Fe^{3+} + 2H_2O + O_2$$

VITAMIN – E (α– TOCOPHEROL)

Vitamin E or Alpha tocopherol is a fat-soluble antioxidant widely distributed in both plant and animal kingdom with many diverse functions in the body. The generic term vitamin E refers to at-least eight structural isomers of α-tocopherol. Among these, α-tocopherol is the well known isomer and possesses the most potent fat soluble antioxidant at high oxygen tension. It is believed to be the first line of defense offering protection to the membrane phospholipids PUFA against peroxidative damage.

Vitamin-E is one of the most important chain breaking antioxidant, it protects the polyunsaturated fatty acids from peroxidative damage by donating hydrogen to the lipid peroxyl radical. Because of the lipophilic property of the tocopherol molecule, vitamin E is the major free radical chain terminator in the lipophilic environment. High levels of tocopherol are found in selected mammalian tissues (e.g. adrenal glands, heart, testes and liver), and this preferential distribution may result from its high lipid solubility. Intracellularly, vitamin E is associated with lipid rich membranes such as mitochondria and endoplasmic reticulum. Thus the antioxidant action of tocopherol is expected to be highly effective in protecting against membrane lipid peroxidation by reacting with lipid peroxyl and alkoxyl radicals.[35]

α– Tocopherol is known as a chain breaking antioxidant because it functions to intercept lipid peroxyl radicals ($LOO^{\bullet}$) and so terminates lipid peroxidation chain reactions. Peroxyl and alkoxyl radicals generated during lipid peroxidation preferentially combine with the antioxidant[23]

$LOO^{\bullet}$ + α – tocopherol OH → LOOH + α – tocopherol+ $O^{\bullet}$

α-tocopherol quenches and reacts with singlet oxygen and could therefore protect the membrane against this species. It also reacts with the superoxide radical.[23]

The chromanol head group of α-tocopherol is very close to the surface of the membrane while the flexible phytyl tail is believed to be approximately aligned with the acyl chains in the interior of the membrane. The close proximity of the chromane head group to the membrane surface is consistent with the acyl chains in the interior of the membrane. The close proximity of the chromane head group to the membrane surface is consistent with the synergistic antioxidant behavior of alpha tocopherol and ascorbic acid.[59] Although the two vitamins are completely sequestered and separated

in their respective lipid and aqueous phases, a very significant extent of inhibition of peroxidation is obtained when both are present.[35]

ASCORBIC ACID (VITAMIN-C)

Vitamin-C or Ascorbic Acid is a water-soluble non-enzymatic antioxidant. Its role as an antioxidant is indicated by its known free radical scavenging action. As a reducing and antioxidant agent, it directly reacts with superoxide and hydroxyl radical and various lipid hydroperoxides. In addition it can restore the antioxidant properties of oxidized vitamin-E, suggesting that a major function of vitamin-C is to recycle the vitamin-E radicals. Ascorbic acid is widely distributed in mammalian tissues, but it is present in relatively high amounts in the adrenal and pituitary glands, lesser amounts are found in the liver, spleen, pancreas and brain. Ascorbic acid serves as both an antioxidant and a proxidant. As an antioxidant, vitamin-C exerts a sparing effect on the antioxidant actions of vitamin-E and selenium. On the other hand, excess amount (≅ mM) may act as a prooxidant in the presence of transition metals Fe^{3+} or Cu^{2+}. Many studies have shown that ascorbate's, Pro-oxidant action, which induces lipid peroxidation, resides in its ability to reduce Fe^{3+} to the Fe^{2+} state. Fe^{2+} is known to be a potent free radical inducer.[35]

Ascorbic acid appears to trap virtually all peroxyl radicals in the aqueous phase before they can diffuse into the plasma lipids. The ability of ascorbate to show antioxidant properties is related to its fast reaction with many reactive oxygen species (peroxyl radicals) and to the fact that the resulting semidehydroascorbate radical is poorly reactive. Enzymatic systems exist invivo to reduce semidehydroascorbate back to ascorbate at the expense of NADH (NADH-semidehydroascorbate reductase enzyme).[60]

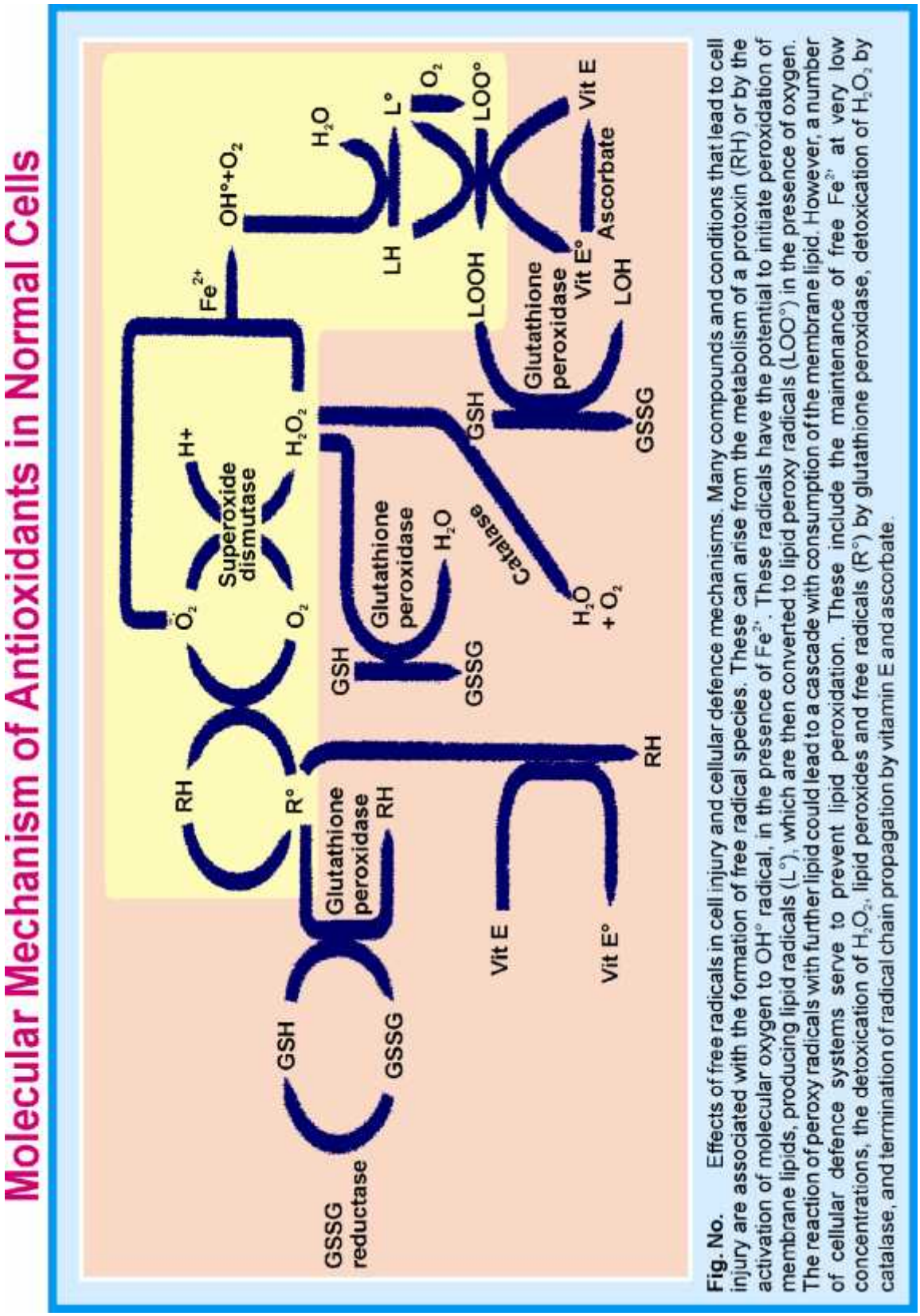


Fig. No. Effects of free radicals in cell injury and cellular defence mechanisms. Many compounds and conditions that lead to cell injury are associated with the formation of free radical species. These can arise from the metabolism of a protoxin (RH) or by the activation of molecular oxygen to OH° radical, in the presence of Fe^{2+}. These radicals have the potential to initiate peroxidation of membrane lipids, producing lipid radicals (L°), which are then converted to lipid peroxy radicals (LOO°) in the presence of oxygen. The reaction of peroxy radicals with further lipid could lead to a cascade with consumption of the membrane lipid. However, a number of cellular defence systems serve to prevent lipid peroxidation. These include the maintenance of free Fe^{2+} at very low concentrations, the detoxication of H_2O_2, lipid peroxides and free radicals (R°) by glutathione peroxidase, detoxication of H_2O_2 by catalase, and termination of radical chain propagation by vitamin E and ascorbate.

CHOLESTEROL:

Cholesterol is essential to life and is a vital constituent of the body. It is a 27 carbon compound with cyclopentanoperhydrophenanthrene ring system.[3] Liver is the principal organ for synthesis of cholesterol. (Fig No 8&9). Other tissues such as adrenal cortex, intestine, skin, ovary, kidney and testis can also synthesize cholesterol. The microsomal and cytosolic fraction of the cell is responsible for the cholesterol synthesis.[61] Daily synthesis is about 1gm out of which 700 mg is endogenous, 300mg is exogenous. Half of the cholesterol is converted into bile acids (400-500mg) trace quantities are utilized for Vit D3, steroid hormone and sex hormone synthesis, remaining is used for the synthesis of cell membrane (as a component of cell membrane).

Normal level of serum cholesterol ranges from 150-220 mg/dl ($2/3^{rd}$ ester and $1/3^{rd}$ free form). Half life of cholesterol is eight days.[62]

Cholesterol level in blood is of primary importance due to its role in the development of atherosclerosis. The OH group in the 3^{rd} position can get esterified to fatty acids to form cholesterol esters. This esterification occurs in the body by transfer of a PUFA moiety by lecithin cholesterol acyl transferase. This has an important role in regulation of cholesterol level in body fluids. It has a modulatory effect on the fluid state of the membrane, is poor conductor of electricity and an insulator of nerve fibers.

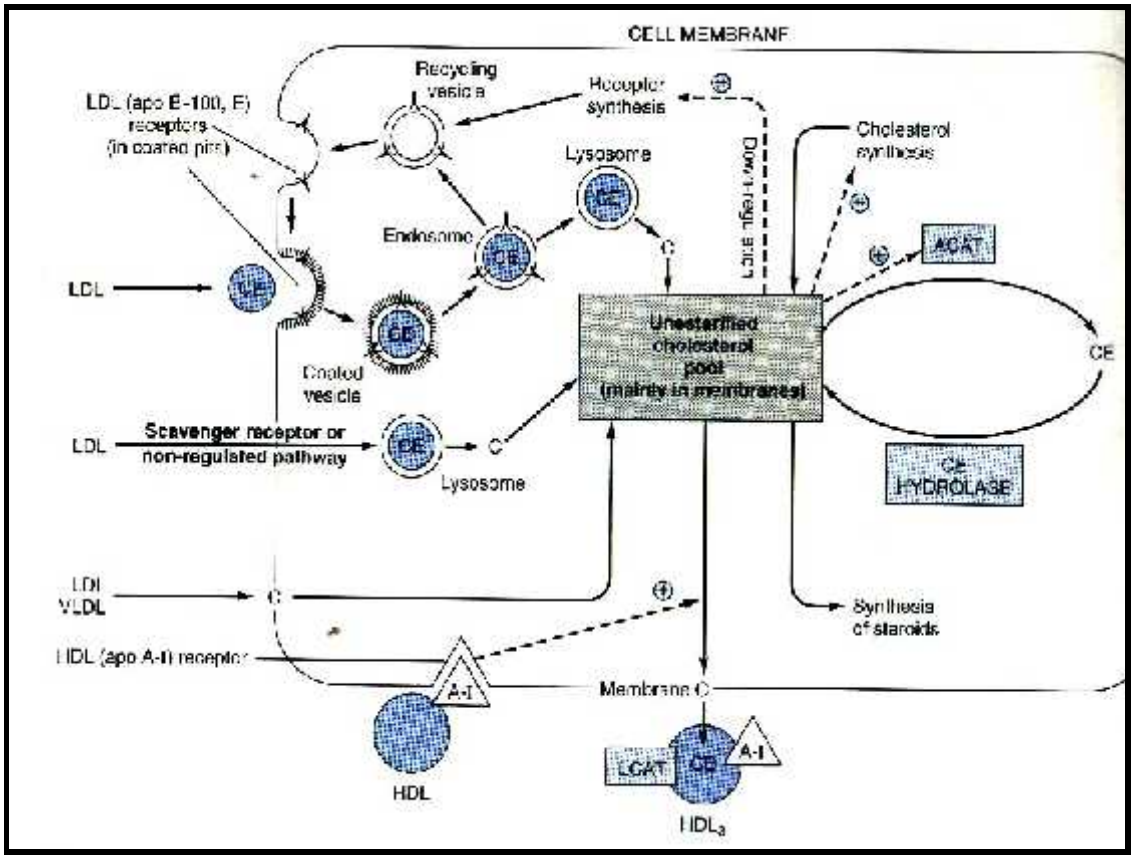


Fig No 8: Factors affecting cholesterol balance at cellular level.[60]

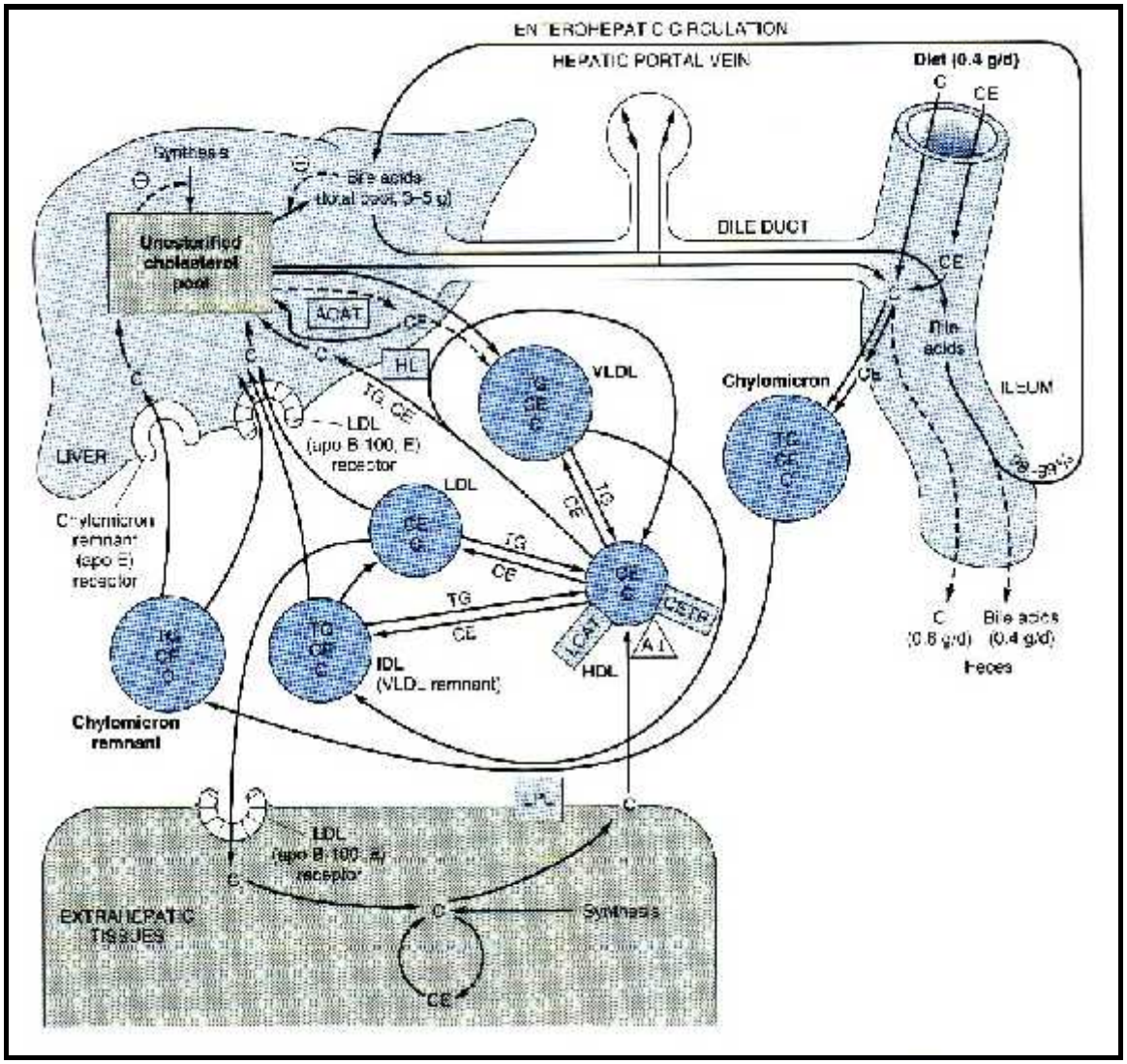


Fig No.9: Transport of cholesterol between the tissues in humans.[60]

TRIGLYCERIDES:

Triglycerides are the esters of fatty acids with glycerol. They constitue about 95% of tissue storage fat and are the predominant form of glyceryl esters found in plasma. Triglycerides are digested in the duodenum and proximal ileum, by the action of pancreatic lipase and colipase (a protein in pancreatic secretion) in the presence of bile salts.[3] They are hydrolyzed to glycerol, monoglycerides and fatty acids. After absorption triglycerides are resynthesized in the intestinal epithelial cells, combines with cholesterol and apo B-48 they form chylomicrons. Chylomicrons are then secreted in to the lymphatic system.

LIPOPROTEINS:

Lipids synthesized in the liver are insoluble and they are transported in the plasma in macromolecular complexes called lipoproteins (Fig. No. 10). These are spherical particles with non-polar lipids in their core and more polar lipids oriented near the surface. They also contain one or more specific proteins called apolipoproteins, that are located on their surfaces.

The association of core lipids with the phospholipids and protein coat is non-covalent, occuring primarily through hydrogen bonding and Vanderwall's forces. This binding of lipid to protein is loose enough to allow the ready exchange of lipids among the plasma lipoproteins and between cell membranes and strong enough to allow the various classes and subclasses of lipoprotein to be isolated and quantified by variety of analytical techniques like ultracentrifugation and electrophoresis (Fig. No. 11&12).

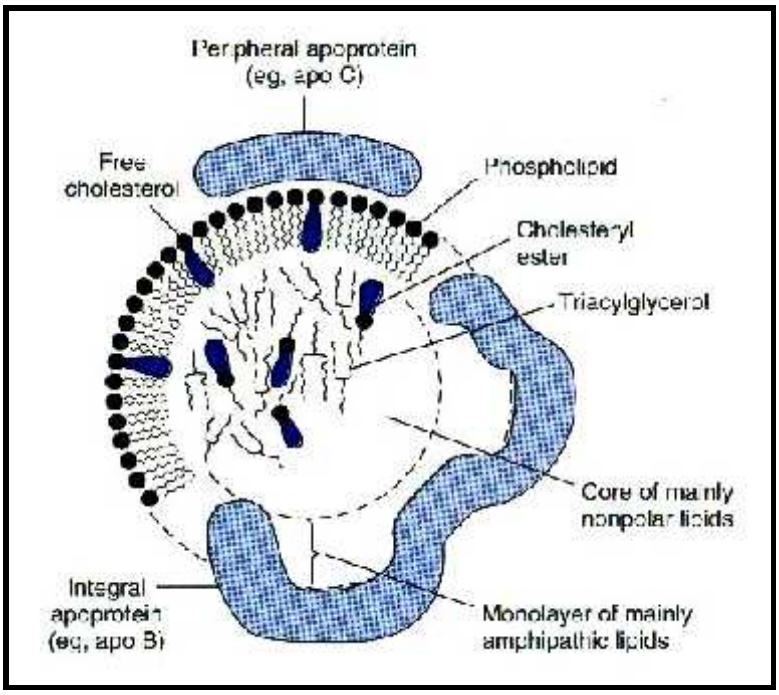


Fig No.10: Generalized structure of plasma lipoprotein.[60]

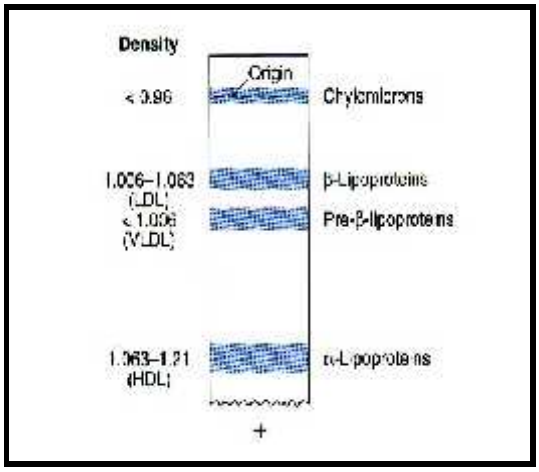


Fig No.11: Separation of Plasma Lipoproteins by gel electrophoresis

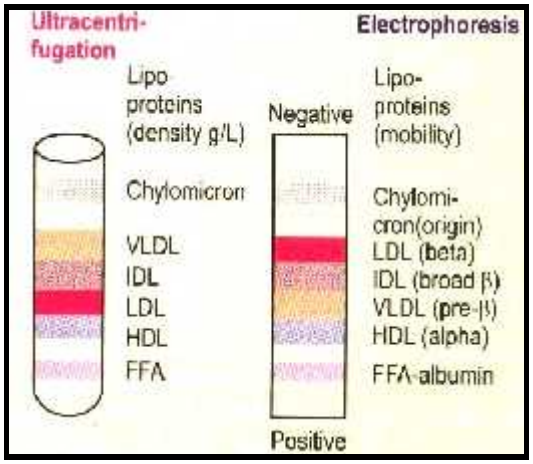


Fig No. 12: Comparison of electrophoretic and ultracentrifuge Separation pattern of lipoproteins

CHYLOMICRONS:

Chylomicrons are the largest of the lipoproteins and the least dense, containing a high proportion of tri-acylglycerols. They are synthesized in the endoplasmic reticulum of epithelial cells that line the small intestine, then move through the lymphatic system and enter the blood stream through the left subclavian vein. The apolipoproteins present in chylomicrons are apo B-48, apoE, and apoC-II. Chylomicrons carry dietary fatty acids to tissues where they will be consumed or stored as fuel. (Fig. No. 13).

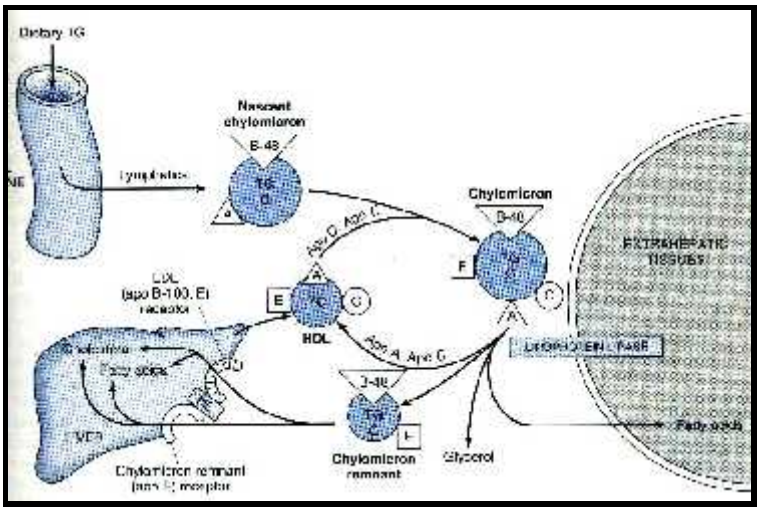


Fig No 13: Metabolic fate of chylomicrons

VERY LOW DENSITY LIPOPROTEINS (VLDL):

They are the major carriers of endogenous triglyceride. They are synthesized in the liver from glycerol and fatty acids and incorporated into VLDL along with hepatic cholesterol, apo-B-100, C-11 and E.

The half life of VLDL in serum is 1 to 3 hours. When they reach the peripheral tissues, apoC-11 activates LPL(Lipoprotein lipase) which liberates fatty acids that are taken up by adipose tissue and muscle. The remnant is now designated as IDL (intermediate density lipoprotein) and contains less of TAG (Triacylglycerol) and more of cholesterol. This IDL contains apoB-100 and apoE. A small part of IDL is taken up by the liver, by receptor mediated endocytosis, helped by B-100 and apoE. The major fraction of IDL further loses triglyceride and apoE, so as to be converted to LDL (low density lipoprotein). This conversion of VLDL to IDL and then to LDL is referred to as lipoprotein cascade pathway (Fig. No. 14).

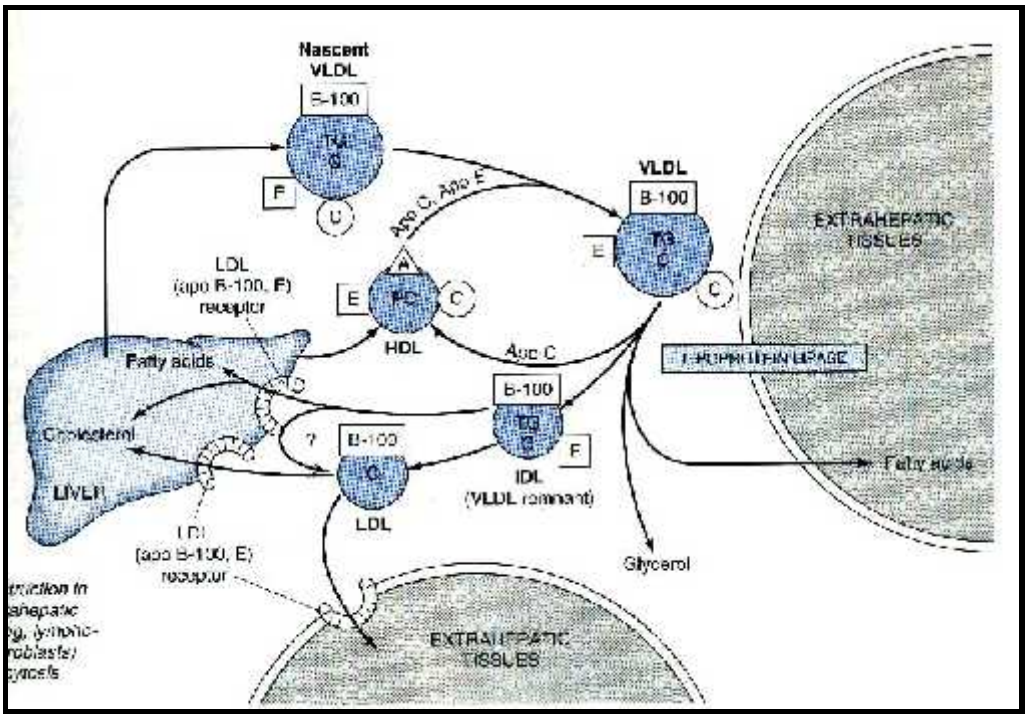


Fig No.14: Metabolic fate of VLDL-C[60]

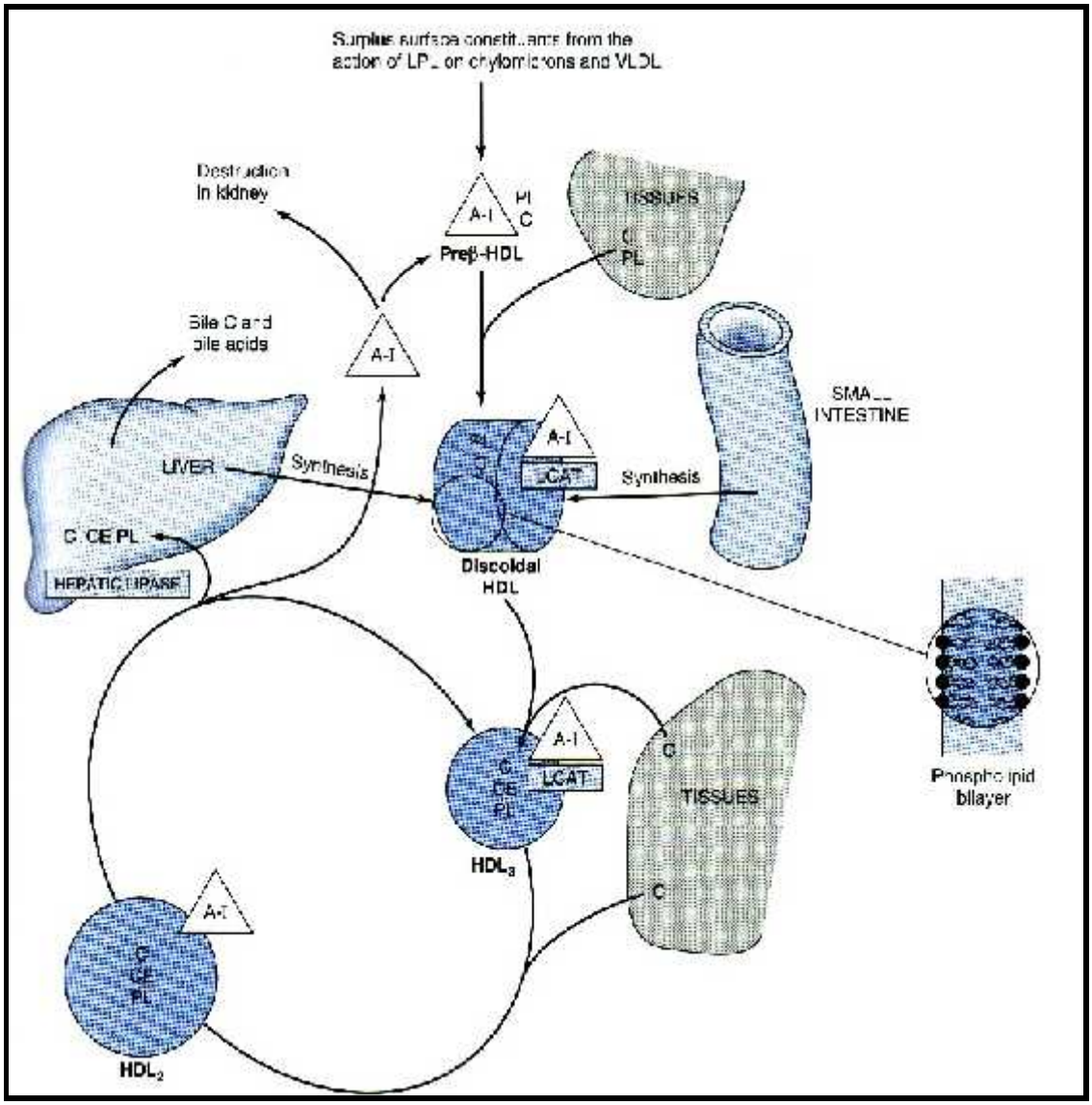


Fig No.15: Metabolic fate of HDL-C[67]

Low Density Lipoprotein (LDL):

The LDL molecules are cholesterol rich lipoprotein molecules containing apo-B-100. About 75% of the plasma cholesterol is incorporated into the LDL particles. Most of the LDL particles are derived from VLDL, but a small part is directly released from liver. The half life of LDL in blood is about 2 days.

High Density Lipoprotein (HDL):

HDL is the main transport form of cholesterol from peripheral tissue to liver, which is later excreted through bile and utilized in the body. The level of HDL-2 in

serum is inversely related to the incidence of myocardial infarction. As it is "antiatherogenic" or "protective" in nature, HDL-2 variety is known as "good cholesterol" as "Highly desirable lipoprotein" in common parlance[3]. (Fig. No.15).

The intestinal cells synthesise components of HDL and release into blood. The nascent HDL in plasma is discoid in shape. The free cholesterol derived from peripheral tissue cells is taken up by the HDL. The apoA-1 of HDL activates LCAT (lecithin cholesterol acyl transferase) present in the plasma.

HDL-l or HDL-C is rich in cholesterol and contain only apoE. It is produced after ingestion of large amount of cholesterol

HDL-3 contains apoA-11 and is further fractionated into 3a, 3b and 3c.

Table 2: Composition of the lipoproteins in plasma of humans.[60]

Fraction	Source	Diameter (nm)	Density	Composition						
				Protein (%)	Total Lipid (%)	Percentages of Total Lipid				
						Triacyl-glycerol	Phosp-holipid	Choles-teryl ester	Cholesterol (Free)	Free Fatty Acids
Chylomicrons	Intestine	90-1000	<0.95	1-2	98-99	88	8	3	1	--
Chylomicron remnants	Chylomicrons	45-100	<0.019	6-8	92-94	80	11	--	4	1
Very low density lipoproteins (VLDL)	Liver (intestine)	30.90	0.95-1.006	7-10	90-93	56	20	15	8	1
Intermediate-density lipoprotein (IDL)	VLDL	25-30	1.006-1.019	11	89	29	26	34	9	1
Low-density lipoprotein (LDL)	VLDL	20-25	1.019-1.063	21	79	13	28	48	10	1
High-density lipoprotein (HDL)										
HDL1	Liver and intestine, VLDL, chylomicrons	20-25	1.019-1.063	32	68	2	53	34	11	--
HDL2		10-20	1.063-1.125	33	67	16	43	31	10	--
HDL3		5-10	1.125-1.210	57	43	13	46	29	6	6
Pre β-HDL[1]		<5	>1.21	70	30	--	83	--	17	--
Albumin-free fatty acids	Adipose tissue	---	>1.281	99	1	0	0	0	0	100

[1]Part of a minor fraction known as VHDL (Very High Density Lipoprotein)

Apo-lipoproteins:

The protein part of lipoprotein is called apolipoprotein (apo-Lp) or apoprotein. Apart from solubilising the lipid part, the protein components have specific function.

One or more apolipoproteins are present in each lipoprotein. According to ABC nomenclature, the major apolipoprotein of HDL (α-Lipoprotein) is designated A. The main apolipoprotein of LDL (β-lipoprotein) is apolipoprotein B and is found also in VLDL and chylomicrons. However, apoB of chylomicrons (B-48) is smaller than apo B-100 of LDL or VLDL. B-48 is synthesized in the intestine and B-100 in the liver.

Apo B-100 is one of the longest single polypeptide chains known, having 4536 amino acids. Apo B-48 (48% as large as B-100) is formed from the same mRNA as apo B-100. Apparently, in intestine, a stop codon that is not present in genomic DNA is introduced by an RNA-editing mechanism that stops translation at amino acid residue 2153 to liberate apo B-48. Apolipoproteins C-I, C-II, and C-III are smaller polypeptides freely transferable between several different lipoproteins. Carbohydrates account for approximately 5% of apoB and include mannose, galactose, fucose, glucose, glucosamine and sialic acid.

Table 3: Apolipoproteins of human plasma lipoproteins.[60]

Apolipo protein	Lipoprotein	Molecular Mass (Da)	Comments and Functions
Apo A-I	HDL, chylomicrons	28,000	Reverse cholesterol transport. Activation of lecithin: cholesterol acyltransferase (LCAT). Ligand for HDL receptor.
Apo A-II	HDL, chylomicrons	17,000	Structure is two identical monomers joined by a disulfide bridge. Inhibitor of apo A-1 and LCAT?
Apo A-IV	Secreted with chylomicrons but transfers to HDL	46,000	Associated with the formation of triacyglycerol-rich lipoproteins. Function unknown. Synthesized by intestine.
Apo B-100	LDL, VLDL, IDL	550,000	VLDL secretion from liver. Ligand for LDL receptor.
Apo B-48	Chylomicrons, chylomicron remnants	260,000	Chylomicron secretion from intestine
Apo C-I	VLDL, HDL, chylomicrons	7,600	Possible activator of LCAT
Apo C-II	VLDL, HDL, chylomicrons	8,916	Activator of lipoprotein lipase
Apo C-III	VLDL, HDL, chylomicrons	8,750	Several polymorphic forms depending on content of sialic acids. Inhibits apo C-II
Apo D	Subfraction of HDL	19,300	May act as lipid transfer protein
Apo E	VLDL, IDL, HDL, chylomicrons, chylomicrons remnants	34,000	Present in excess in the β-VLDL of patients with type III hyperlipoproteinemia. The sole apoprotein found in HDLc of diet induced hypercholesterolemic animals. Ligand for chylomicron remnant receptor in liver and LDL receptor.

MATERIALS AND METHODS

Source of data:

The present study comprises of 50 clinically diagnosed and ECG confirmed cases of acute myocardial infarction admitted to the intensive cardiac care unit of KLE Society's Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. The study was undertaken between September 2007 to August 2008. The cases were classified into two groups. Group I included 25 cases of type II diabetes mellitus and Group II consisted of 25 cases of hypertension. All the patients were in the age group of 40-60 years. Age and sex matched 50 healthy individuals served as controls.

Criteria for the selection of the cases:

1) Inclusion criteria: Clinically diagnosed and ECG confirmed cases of acute myocardial infarction.

2) Exclusion criteria:
   a) Angina pectoris
   b) Aortic dissection
   c) Infective hepatitis
   d) Renal failure
   e) Pulmonary tuberculosis, pneumonia, pneumothorax & massive pulmonary embolism
   f) Rheumatoid arthritis
   g) Costochondritis (Tietze's syndrome)
   h) Chronic smokers and alcoholics
   i) Pregnancy
   j) Peptic ulcer

Informed consent was taken from all the cases and the study was approved by the ethical and research committee of JNMC, Belgaum.

Collection and storage of blood sample:

10ml of blood was collected from the patients immediately after admission. Samples were also collected from the controls, under aseptic precautionary measures by using disposable syringe. Out of this 1ml of whole blood was used for estimation of malondialdehyde and 1ml was used for the preparation of hemolysate. Enzymes like catalase, glutathione reductase and superoxide dismutase were analysed from the hemolysate within one hour. 4ml of blood was centrifused immediately and plasma was obtained for estimating vitamin E and vitamin C. Remaining 4ml of blood was allowed to stand for sometime and the serum was separated and used for estimating total cholesterol, HDL and triglycerides.

Methods of assay

A) Whole blood

- Malondialdehyde (Thiobarbituric acid method)

B) Hemolysate

I) Haemoglobin (Drabkin's method)

II) Enzymatic antioxidants

a. Superoxide dismutase (Misra and Fridorich)

b. Glutathione reductase (Beutler's E method)

c. Catalase (Beutler's E method)

C) Plasma

I. Vitamin E (Quafie et al, Baker and Frank)

II. Vitamin C (Evelyn and Melloy method)

D) Serum

i. Total cholesterol (Carr and Drector method)

ii. HDL (Lopez-Virella ML et al method)

iii. Triglyceride: Classical Method (Modification of Van Handle and Zilversmith)

E) By calculation – using Friedewald's formula

I. LDL

II. VLDL

The values of blood glucose and blood pressure were taken and the cases were divided into Group I and Group II.

STATISTICAL ANALYSIS:

Statistical analysis was carried out by using student's t – test (unpaired).

ESTIMATION OF MALONDIALDEHYDE (MDA) IN WHOLE BLLOD:[63]

Principle:

The reaction depends on the formation of pink coloured complex between malondialdehyde and thiobarbituric acid (TBA), having an absorption of maximum at 532 nm.

Thiobarbituric acid reagent:

- 75 ml thiobarbituric acid

- 15 gm trichloroacetic acid
- 2.08 ml – 0.2 N HCI

All were mixed and volume made up to 100 ml with distilled water.

Procedure:

	Blank (ml)	**Test (ml)**
Whole blood	-	0.75
Distilled water	0.75	-
Thiobarbituric acid reagent	3	3

- Keep in boiling water bath for 15 minutes
- Cool, centrifuge for 10 minutes at 10,000 r.p.m.
- Read absorbance of supernatants of blank and test immediately at 535 nm

Calculation:

Malondialdehyde (nano moles / ml)

$$= \frac{\text{Absorbance of test} \times \text{total volume}}{\text{Nanomolar extension coefficient} \times \text{Sample volume} \times 100}$$

$$= \frac{\text{Absorbance of test} \times 3.75}{1.56 \times 10^5 \times 0.75 \times 100}$$

$$= \frac{\text{Absorbance of test} \times 3205}{100}$$

PREPARATION OF (RBC) HEMOLYSATE

Isolation of Red Blood Cells: [64]

Most of the enzyme activities in red cells are lower than those in white blood cells and platelets, and hence it was of extreme importance to remove virtually all platelets and WBCs. In order to isolate RBCs the whole blood was filtered through a column of α-cellulose and microcrystalline cellulose mixture.

α – Cellulose and microcrystalline cellulose in 1:1 (W/W) was mixed with isotonic (9.0 gm/L) sodium chloride solution. 5ml plastic disposable syringe without barrel was taken. It was placed in vertical position with the outlet pointing downwards. A small piece of filter paper was placed at the bottom of the syringe. The well – mixed cellulose slurry was poured to the 2-ml mark. The bed was washed with 5-ml isotonic sodium chloride and 1 ml of whole blood was allowed to flow through the column. To ensure efficient removal of WBCs and platelets, the volume of cellulose mixture used was at least twice as that of the blood sample.

The effluent was collected into a centrifuge tube. The saline suspended red cells were washed twice in at least 10 volumes of ice-cold isotonic sodium chloride. After washing, the packed cells were resuspended in isotonic sodium chloride to give an approximately 50% suspension (1:1) dilution). This suspension was subjected to hemolysis.

LYSING OF THE RBCS:[65]

Reagents:

Stabilizing solution: 2.7 mM EDTA (pH 7.0) and 0.7mM β- mercaptoethanol: This solution was prepared by dissolving 100 mg of disodium salt of EDTA in D/W and

5µl of β- mercaptoethanol (Merck) were added to it. Final volume was made to 100 ml with D/W.

Procedure:

In order to prepare the hemolysate, 1 volume of the RBC suspension was mixed with 9 volumes of the stabilizing solution. The hemolysate was frozen rapidly at – 20^0C to – 25^0C in a freezer. Then it was thawed in a water bath at 20^0C to 25^0C. This hemolysate was then ready for the assay. The hemoglobin estimation was performed on hemolysate using Drabkin's reagent, in order to express the enzyme activities per gram of hemoglobin of the hemolysate. The red cell suspension and its hemolysate were prepared on the day of the assay.

The hemolysate was used for the following enzyme activities-

a) Superoxide dismutase (SOD)

b) Catalase

c) Glutathione reductase (GR)

DETERMINATION OF HEMOGLOBIN[66]

Principle:

Drabkins reagent contains potassium cyanide and potassium ferricyanide Hemoglobin reacts with ferricyanide to form methemoglobin, which is converted to stable cyanmethemoglobin (HiCN) by the cyanide. The intensity of the colour is proportional to hemoglobin concentration and is compared with a known cyanomethemoglobin standard at 540 nm (green filter).

Reagents:

1. Drabkin's reagent: The prepared reagent was purchased (Span Diagnostics).

2. Cyanmethemoglobin standard, (cyanmeth-Hb standard) 15g%: The prepared standard was purchased (Span Diagnostics)

Procedure:

A set of tubes was prepared as follows:

	Bank (ml)	**Standard (ml)**	**Test (ml)**
Drabkin's reagent	5.0	---	5.0
Cyanmethemoglobin standard	----	5.0	----
Unknown	----	----	0.02

The contents in the test were mixed thoroughly and optical density of test and standard (15g %) were measured at 540 nm against blank (Drabkin's reagent)

Calculation:

$$\text{Hemoglobin g\%} = \frac{\text{ODtest}}{\text{ODstd}} \times 15$$

PERFORMANCE OF THE ENZYME ASSAYS FROM 1:20 HEMOLYSATE

SUPEROXIDE DISMUTASE[67]

Principle:

Epinephrine can be auto-oxidized to adrenochrome by superoxide radicals. Maximum auto-oxidation of epinephrine takes place at pH-10.2. The ability of superoxide dismutase to inhibit the auto-oxidation of epinephrine to adrenochrome at pH 10.2 has been used as the basis for the assay of this enzyme. In this method epinephrine acts both as the source of superoxide radical ($O_2^{\bullet -}$) and as the detecting system giving adrenochrome which can be monitored at 480 nm.

Reagents:

1) Chloroform.

2) Ethanol.

3) Sodium carbonate/ bicarbonate buffer. 0.05 M, pH 10.2;

This was prepared as follows.

a) Sodium carbonate (Na_2CO_3).0.2 M: 2.12g of anhydrous Na_2CO_3 was dissolved in small quantity of D/W and volume made to 100ml with D/W.

b) Sodium bicarbonate ($NaHCO_3$), 0.2M: 1.68g of $NaHCO_3$ were dissolved in small quantity of D/W and volume was made to 100ml with D/W

Solution (a) 33ml was mixed with solution (b) 17ml in small quantity of D/W, pH of the solution was adjusted to 10.2 and volume was made to 200 ml with D/W.

4) DL-(α) Epinephrine, 3 x 10^{-2} M: 55 mg of Dl-(α) Epinephrine were dissolved in 3ml of D/W. To dissolve epinephrine completely minimum amount of 1NHC1 was added and volume was made to 10ml with D/W.

5) EDTA (Na_2EDTA), 15 x 10^{-3} M: 49 mg of Na_2EDTA were dissolved in minimum quantity of D/W and volume was made to 10ml with D/W.

Procedure: Preparation of hemoglobin free filtrates:

Chloroform, ethanol and water were chilled before use. In a test tube, chloroform (0.125ml), ethanol (0.25ml) and D/W (0.8ml) were added. To this mixture, 0.2ml of cold hemolysate was transferred. The suspension was subjected to vortex agitation for 2 minutes by transferring the tube intermittently to ice bath. The precipitate was separated by centrifugation at 15,000 rpm at 4^0C for 10 minutes and the supernatant was used for the assay.

SOD Enzyme assay:

For the assay the final reaction mixture in control-contained epinephrine ($3x10^{-4}$M), EDTA ($1x10^{-4}$M) and carbonate buffer (0.05M). In a series of tests, in addition to the above reagents, varying amounts of chloroform-ethanol extracts were added.

Control and tests were setup as under:

	Control	$Test_1$	$Teast_2$	$Test_3$	$Test_4$	$Test_5$	$Test_6$
Carbonate buffer, 0.05M pH(10.2) (ml)	2.95	2.90	2.85	2.80	2.75	2.70	2.65
Chloroform-ethanol extract (µl)	----	50	150	150	200	250	300
EDTA, 15 x 10^{-3}M (ml)	0.02	0.02	0.02	0.02	0.02	0.02	0.02

To control tube 0.03ml of epinephrine ($3x10^{-2}M$) was added and after 90 secs (the lag phase) the auto-oxidation of epinephrine was assayed by measuring the optical densities at 0 minute and after 3 minutes, at 480nm.

To $Test_1$ 0.03ml of epinephrine was added and after 90 seconds, optical densities were measured at 0 minute and after 3 minute at 480 nm.

In a similar fashion the assay was performed by adding 0.03 ml of epinephrine one by one to each tube and after lag phase of 90 seconds optical densities were measured at 0 minute and after 3 minutes at 480 nm.

Calculations:

SOD activity was determined in terms of its inhibition of auto oxidation of epinephrine to adrenochrome.

One enzyme unit is the amount of protein required to inhibit the auto-oxidation of epinephrine by 50% under standard conditions of assay.

Percent inhibition was calculated from the optical density of each tube with varying amounts of enzyme (red cell extract) and optical density of the control without enzyme.

$$\text{Percent inhibition} = 100 - \frac{\Delta ODs}{\Delta ODc} \times 100$$

Where ΔODs: Difference in optical density of sample in 3 minutes.

ΔODc: Difference in optical density of control in 3 minutes.

A graph percent inhibition against amount of red cell extract was plotted as shown in the figure 16, to determine 50% inhibition of epinephrine oxidation. The amount of extract, which gave 50% inhibition, as determined from the graph corresponds to 1 unit of SOD activity.

V µl = 1 unit of SOD activity

Units of SOD activity per 100 ml of red cell extract / X gm of Hb = $\frac{100 \times 1000}{V\ \mu l}$ units

Units of SOD activity / gm of hemoglobin = $\frac{100 \times 1000}{V\ \mu l} \times \frac{1}{x}$

NB:for each sample the graph was plotted to determine the 50% inhibition as shown below.

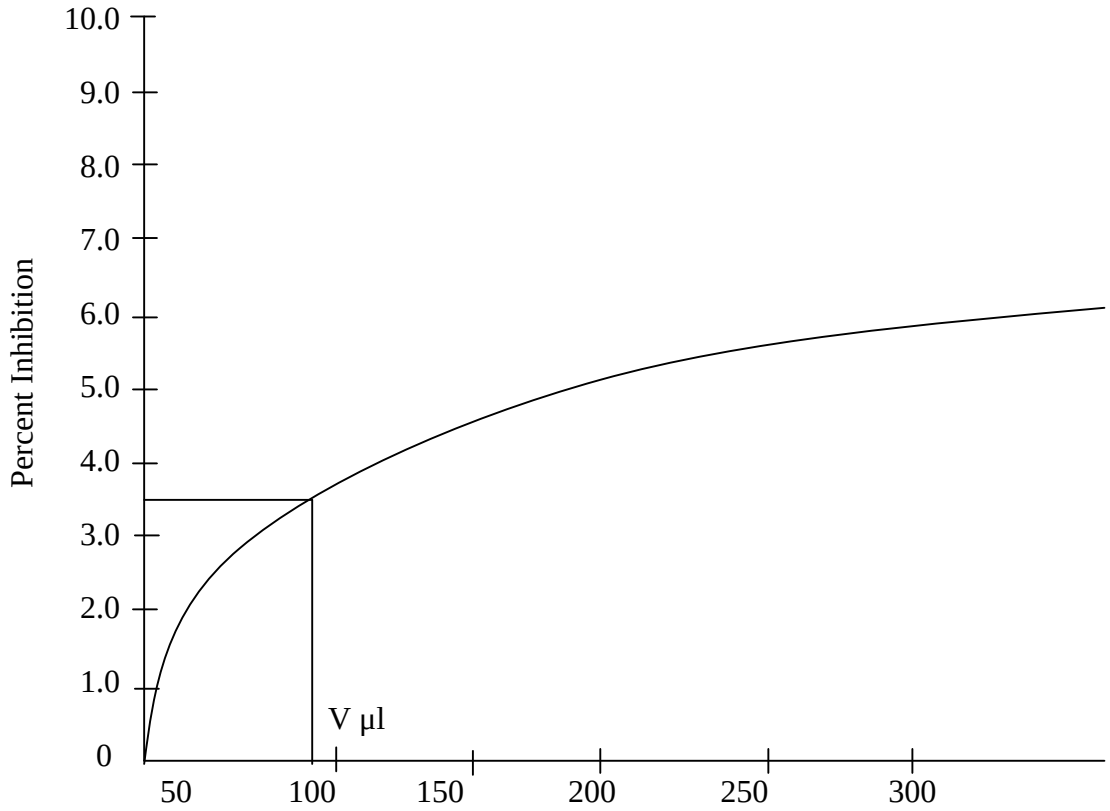


Fig. No 16: Determination of SOD Activity Red cell extract (rl)

Calculation:

The number of enzyme units per ml:

$$A = \frac{\Delta OD \times Vc}{\varepsilon \times N \times V_H}$$

Where

ΔOD: Change in optical density per minute

$$\Delta OD: \frac{Test - (Blank_1 + Blank\ )}{10 \text{ minutes}}$$

Vc : The volume of the cuvette in ml = 1ml

ε : The millimolar extinction coefficient of the NADPH = 6.22

N: The number of molecules (1) of NADPH converted per molecule of t-BHP consumed.

V_H : The volume of hemolysate added to the cuvette in ml = 0.01ml

The enzyme activity in international unit / g Hb

$$E = \frac{A \times 100}{Hb}$$

A: The number of enzyme units /ml

Hb: The grams of hemoglobin per 100ml of the hemolysate.

GLUTATHIONE REDUCTASE[68]

Principle:

Glutathione reductase (GR) catalyzes the reduction of oxidized glutathione (GSSG) by NADPH to reduced glutathione.

$$NADPH\ (NADH) + H^+ + GSSG \xrightarrow{GR} NADP^+\ (NAD^+) + 2GSH$$

The activity of this enzyme is measured by following the oxidation of NADPH (NADH) spectrophotometrically at 340nm. Glutathione reductase is a flavin enzyme and it has been found that it is not fully activated by FAD in normal hemolysates. Complete activation of apoenzyme, requires the preincubation of the enzyme with

FAD. This must be done before GSSG or NADPH is added to the reaction system, since these seem to interfere with activation of the enzyme by FAD.

Reagents:

1) Tris-HCI; 1M, EDTA: 5mM, pH 8.0: It was prepared as described earlier.

2) Flavin adenine dinucleotide (FAD), 10 µM: 0.8 mg of sodium salt of FAD (SRL, Mwt-829.52) was dissolved in 100 ml of D/W.

3) Glutathione oxidized (GSSG), 0.033M: 20.2 mg of GSSG (SRL, Mwt-612.7) were dissolved in 1ml of D/W.

4) Nicotinamide adenine dinucleotide phosphate reduced (NADPH) 2nmM: It was prepared as described earlier.

Procedure:

The following reagents were added to the cuvettes volume of 1ml

	Blank (µl)	**Test (µl)**
Tris HCI; 1M EDTA, 5mM, pH 8.0	50	50
1:20 hemolysate	10	10
D/W	790	690
FAD 10 µM	100	100
Tubes were incubated at 37^0C for 10 minutes		
GSSG, 0.033M	--	100
Tubes were incubated at 37^0C for 10 minutes		
NADPH, 2Mm	50	50

The decrease of optical density was measured at 340 nm at 37^0C of the test against the blank.

Calculations:

The number of enzyme units per ml:

$$A = \frac{\Delta OD \times Vc}{\varepsilon \times N \times V_H}$$

Where

ΔOD: Change in optical density per minute

Vc : The volume of the cuvette in ml = 1ml

ε : The millimolar extinction coefficient of the NADPH = 6.22

N: The number of molecules (1) of NADPH converted per molecule of GSSG consumed.

V_H : The volume of hemolysate added to the cuvette in ml = 0.01ml

The enzyme activity in international units / g Hb

$$E = \frac{A \times 100}{Hb}$$

A: The number of enzyme units /ml

Hb: The grams of hemoglobin per 100ml of the Hemolysate.

CATALASE: [68]

H_2O_2 Oxidoreductase, ECI 11.1.6

Principle:

Catalase catalyses the breakdown of hydrogen peroxide according to the following reaction.

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

The rate of decomposition of H_2O_2 by catalase is measured spectrophotometrically at 230 nm. Since hydrogen peroxide absorbs light at this wavelength.

Ethanol is added to stabilize the hemolysate by breaking down the "Complex II" of catalase and hydrogen peroxide.

Reagents:

1) Tris-HCI, 1M: EDTA, 5mM; pH 8.0; 12.1g of Tris and 168mg of disodium salt of EDTA were dissolved in 80ml of D/W and pH was adjusted to 8.2 with concentrated hydrochloric acid and further till 8.0 with 1N Hcl and final volume was made to 100ml with D/W.

2) Phosphate buffer, 1M, pH 7.0: 100ml of 1M Na_2HPO_4, H_2O_2 (17.79 g %) solution was adjusted to pH 7.0 with 1M NaH_2PO_4, $2H_2O$ (15.6g %) solution.

3) Hydrogen peroxide (H_2O_2); 10mM: Optical density of 2.7ml of 1; 10 diluted 1M-phosphate buffer, pH 7.0 was measured at 230 nm as OD1. Three microliters of 1:100 diluted 30% H_2O_2 solution were added and optical density was measured as OD2. Since the millimolar extinction coefficient of H_2O_2 at 230 nm is 0.071, the H_2O_2 concentration (C) of the 1:100 diluted peroxide solution is 141 (OD2 - OD1) mM. To obtain H_2O_2 10mM for the assay, 1ml of 1:100 diluted H_2O_2 was further diluted to C/10ml with D/W.

4) 1:2000 hemolysate with ethanol: 1:20 hemolysate prepared in β-mercaptoethanol EDTA stabilizing solution. Solution was further diluted to 1:100 with EDTA stabilizing solution. Twenty microliters absolute ethanol were added per milliliter of dilute hemolysate (to breakdown "Complex II" which may be present).

Procedure:

A set of test tubes was prepared as follows:-

	Blank (ml)	**Test (ml)**
Tris HC1; 1M EDTA, 5mM, pH 8.0	0.15	0.15
H_2O_2 10mM	----	2.70
D/W	2.79	0.09
Tubes were incubated at 37^0C fro 10 minutes		
1:2000 hemolysate	0.06	0.06

Calculations:

The number of enzyme units per ml is

$$A=\frac{\Delta OD \times Vc}{\varepsilon \times N \times VH}$$

Where

ΔOD: Change in optical density per minute

Vc : Volume of the cuvette in ml

ε : The millimolar extinction coefficient of the hydrogen peroxide = 0.071

N : The number of molecule (1) of hydrogen peroxide converted per molecule of substrate (H_2O_2) consumed

V_H : The volume of hemolysate in ml = 6×10^{-4}

The enzyme activity in international units/g Hb is

$$E = \frac{A \times 100}{Hb}$$

A : Number of enzyme units per ml

Hb : The grams of hemoglobin per 100 ml of the hemolysate

ESTIMATIONS FROM PLASMA[69]

α- TOCOPHEROL

Principle:

This method is based on the Emmerie Engel reaction. Xylene extract of plasma containing α-tocopherol when reacts with ferric chloride, reduces ferric ions to ferrous ions. The ferrous ions then react with α, α' – dipyridy1 to give a red colored complex which is measured at 520 nm. Carotenoids, which are also extracted into xylene, are estimated by their absorbance at 460 nm and a correction is applied at 520 nm. The carotenoid absorption at 520 nm is 29% of absorption of 460 nm.

Reagents:

1) Absolute ethanol, aldehyde free

2) Xylene

3) n-propanol

4) α, α' Dipyridy1 reagent: 120 mg %: 120 mg of dipyridy1 were dissolved in small quantity of n-propanol and volume was made to 100 ml with n-propanol.

5) Ferric chloride reagent 120 mg %: 120 mg of ferric chloride hexahydrate ($FeCl_3.6H_2O$) were dissolved in small quantity of absolute ethanol and volume was made to 100 ml with absolute ethanol.

6) α- Tocopherol, stock standard 280 mg %: 280 mg of α – tocopherol were dissolved in small quantity of absolute ethanol and volume was made to 100 ml with absolute ethanol.

7) α– Tocopherol. Working standard: 1.4 mg %: 0.1 ml of the stock standard of α – tocopherol was diluted to 20 ml with absolute ethanol

Procedure:

A set of test tubes was prepared as follows:

	Blank (ml)	**Standard (ml)**	**Test (ml)**
Distilled water	1.5	-	-
Std α – tocopherol	-	1.5	-
Plasma	-	-	1.5
Absolute ethanol	1.5	1.5	1.5
Xylene	1.5	1.5	1.5

Tubes were stoppered and mixed on a vortex mixer for 2 minutes. After centrifugation at 2000 rpm for 10 minutes, 1.0 ml of xylene layer was withdrawn from each tube into separate set of corresponding tubes. To all the tubes 1.0 ml of α, α' – dipyridy1 reagent was added. The contents of the tubes were mixed and absorbance of test and standard was read at 460 nm, against blank. Beginning with the blank, to all tubes 0.4 ml of ferric chloride reagent was added and contents were mixed for 30 seconds. The absorbance was read exactly after 90 seconds of the addition of ferric chloride reagent. The test and standard were read at 520 nm against blank.

Calculation:

Plasma levels of α- tocopherol are expressed as mg %

Concentration of α – tocopherol (mg %) =

$$\frac{ODT_{520} - (0.29 \times OD\ T_{460})}{OD\ S_{520} - (0.29 \times OD\ S_{460})} \times C =$$

Where

OD T_{520} : Optical density of test at 520 nm

OD T_{460} : Optical density of test at 460 nm

OD S_{520} : Optical density of standard at 520 nm

OD S_{460} : Optical density of standard at 460 nm

C : Concentration of standard α – tocopherol in mg %

ASCORBIC ACID[70]

Principle:

When ascorbic acid reacts with 2, 6-dichlorophenol indophenols, reduced 2, 6-dichlorophenol indophenol is formed which is colorless. Decrease in color is proportional to the concentration of ascorbic acid present in the solution. Decrease in the optical density is measured at 520nm and concentration is calculated from standards treated similarly.

Reaction:

2, 6-dichlorophenol indophenol (Oxidized form) + Ascorbic acid ⟶

2, 6-dichlorophenol indophenol (Reduced form) + Dehydroascorbic acid

Reagents:

1) 10% sodium tungstate: 10 g of sodium tungstate were dissolved in D/W, and the volume was made up to 100 mL.

2) 2/3 N sulphuric acid. Take 18.7ml concentrated H_2SO_4. Mix with distilled water make the volume up to 1 liter with D/W.

3) Metaphosphoric acid solution; 5%: 5g of metaphosphoric acid (Robert Johnson) is dissolved in 100ml of D/W without heating. Solution should be prepared weekly. Store in refrigerator.

4) 2, 6 dichlorophenol indophenol solution: 13mg of 2, 6 dichlorophenol indophenol (Loba GR) and 3g of anhydrous sodium acetate trihydrate (Qualigens SQ), were dissolved in 1 liter of D/W. 9ml of this reagent and 1 ml of metaphosphoric acid (reagent 1) were mixed to check the pH which should be around 3.5-3.6 (pH can be adjusted by varying the amount of sodium acetate in the reagent).

5) Ascorbic acid stock standard, 100mg%: 100mg of L-Ascorbic acid was dissolved in 100ml of metaphosphoric acid.

6) Ascorbic acid working standard, 1 mg%: 1ml of the stock standard ascorbic acid was diluted to 100ml with metaphosphoric acid.

Procedure:

In a clean dry test tube, 1 ml of plasma, 3ml of 5% metaphosphoric acid, 0.5ml of sodium tungstate and 0.5ml of 2/3N sulfuric acid were taken. The contents were mixed and filtered after 5 minutes.

Three clean dry test tubes labeled as Blank, Standard and Sample were taken, and following reagents were added as shown as follows.

	Blank (ml)	**Standard (ml)**	**Sample (ml)**
5% Metaphosphoric acid	0.3	-	-
Ascorbic acid standard	-	0.3	-
Plasma	-	-	0.3
2,6-dichlorophenol indophenol	2.7	2.7	2.7

Reading was taken at 520nm against D/W using a double beam Spectrophotometer.

Calculations:

Amount of ascorbic acid in mg% =

$$\frac{\text{OD of Blank} - \text{OD of sample}}{\text{OD of Blank} - \text{OD of Standard}} \times \text{concentration of standard (in mg \%)}$$

ESTIMATION OF SERUM TOTAL CHOLESTEROL [71]:

(Method of Carr and Drekter)

Reagents:

1. Glacial acetic acid, A.R.
2. Acetic anhydride, A.R.: Keep tightly stoppered
3. Sulfuric acid, A.R.:36N Keep tightly stoppered
4. Cholesterol standard: Dissolve 200mg of purified cholesterol in approximately 50ml glacial acetic acid; then dilute to 100ml exactly.

5. Sulfuric acid – acetic acid reagent; 1:1 (v/v). Slowly, and carefully, pour 100ml concentrated sulfuric acid, preferably pre cooled in a refrigerator, into a 500ml, pyrex flask containing 100ml of glacial acetic acid. Mix the contents by gentle rotation until the addition is complete; then allow the mixture to cool to room temperature before using. If kept tightly stoppered, this reagent is stable at room temperature for at least 6 months.

6. Dehydrating reagent; mix equal volumes of reagent 1 (acetic acid) and reagent 5 (sulfuric-acetic acids). Make fresh daily.

Procedure:

For each serum or plasma sample to be analyzed, set up two tubes, one of which will serve as a blank. Each run of analyses should also include a tube for a standard sample and for a reagent blank. Add in the proper order the indicated volume (ml) of the following materials. Protect all tubes from exposure to intense light.

		Reagent blank	**Standard**	**Unknown serum**	**Serum blank**
1.	Standard solution	--	0.2	---	---
2.	Serum	---	---	0.2	0.2
3.	Water	0.2	0.2	---	---
4.	Acetic acid	0.8	0.6	0.8	0.8

5. Allow all of the tubes to stand at room temperature for 1 to 2 minutes. Make up volume with the addition of the acetic acid. Next add 4.0ml of acetic anhydride (No.2).

6. Centrifuge all the serum containing tubes for 5 minutes at 2000 rpm, and then decant the supernatant solution as completely as possible into clean, dry tubes properly labeled. Discard protein precipitate.

7.	Acetic acid	--	--	--	0.1
8.	Dehydrating reagent (No.6)	0.1	0.1	0.1	---

9. Mix the tubes by rotation. The contents should become hot in 1 to 2 minutes; if not, add a second drop of the dehydrating reagent, then place al the tubes in a water bath at 25^0C. Allow them to remain there for 5 to 10 minutes.

10. At 1 minute intervals, add 1 ml of the dehydrating reagent (No.6) to all tubes except the serum or plasma blanks which receive 1 ml of acetic acid (No.1). Begin with the reagent blank. After addition, mix the contents well; then return them to the water bath for 20 minutes before measuring the absorbance. Reading should start with the reagent blank, then be made in serial order at 1 minute intervals. Set the photometer at 620 nm and adjust to zero with reagent blank solution.

Calculation: mg cholesterol/100ml serum= $\frac{A\text{unknown} - A\text{serum blank}}{A\text{standard}}$ x 200 x 1.01

ESTIMATION OF HDL-CHOLESTEROL[72]:

(Lopez-Virella ML et al)

1. Into 100mm test tubes or 15 mm tubes or 15 mm conical centrifuge tubes, add 0.5ml of serum of the patient and control, seperately.

2. Add 0.100 ml of sodium phosphotungstic acid solution followed by 0.025 ml of 2mol/L $MgCl_2$mix.

After 10 min at room temperature, centrifuge the tubes for 15 min

Aspirate the supernatant into a clean, labeled test tube.

Perform cholesterol determination by Carr-Drekter method

Calculations:

Generally a 100 mg/dl or 50 mg/dl cholesterol standard should be used for HDL-cholesterol determinations.

Absorbance of unknown* Concentration of standard

Absorbance of standard = mg/dl HDL – cholesterol in unknown (mg/dl)

ESTIMATION OF TRIGLYCERIDE[73]

Procedure: Classical Method (Modification of Van Handle and Zilversmit)

Principle:

This manual method extracts triglycerides with chloroform while adsorbing phospholipids with zeolite. An aliquot of the chloroform extract is added to alcoholic potassium hydroxide to saponify the triglycerides to glycerol and free fatty acids. Glycerol is then oxidized with periodate to formaldehyde, which is measured colorimetrically after reaction with chromotropic acid. The method is practical for routine testing if large batches of samples are not anticipated.

Reagents:

Extraction chemicals:

Chloroform: reagent grade. Keep in dark bottle

Zeolite: 80 mesh (W.A. Taylor, Co., 7300 York Rd., Baltimore, MD) heat for 4 hours at 125^0C to activate.

Alcoholic potassium hydroxide

Stock (2%, 356 nmol/L). Dissolve 2g of potassium hydroxide in 95% ethanol; dilute to 100mL with 95% ethanol. This is stable for 1 month at ambient temperature.

Working (0.4%, 71 nmo1/L). Dilute 10 mL of stock solution to 50mL with 95mL. ethanol. Prepare fresh daily.

Sulfuric acid (0.2 N, 0.1 mol/L): Dilute 3 mL of concentrated sulfuric acid to 500mL with reagent-grade water. This is stable for 6 months at room temperature.

Sodium metaperiodate (0.05 mol/L): Dissolve 1.07g of sodium metaperiodate in reagent-grade water. Dilute to 100mL. This is stable for 1 month at 4^0C

Sodium arsenite (0.5mol/L): Dissolve 6.5g of sodium arsenite in reagent-grade water; dilute to 100mL. This is stable for 6 months at room temperature.

0.2% chromotropic acid (6.14 mmol/L): Dissolve 2g of chromotropic acid (or 2.24g of its sodium salt) in 100mL of reagent-grade water. Add separately 600mL of concentrated sulfuric acid slowly with mixing to 300mL of reagent-grade water cooled in ice. When it is cool, add the diluted sulfuric acid solution to the chromotropic acid solution. Store in brown bottle. Prepare fresh every 2 to 4 weeks.

Triglyceride stock standard: 500mg/L (5.65 mmol/L). Dissolve 500mg of triolein in chloroform. Dilute to 100mL with chloroform in a glass-stopped volumetric flask. Store tightly sealed at 4^0C. Stable for 3 months.

Triglyceride working standard: 500mg/L (565 µmol/L). Dilute 1 mL of stock standard to 10mL with chloroform. This is stable for 1 week. Store tightly sealed at 4^0C.

Assay:

Equipment: shaker; water bath at 65^0C, water bath at 100^0C; spectrophotometer with band pass $\leq$ 10nm and capable of reading at 550 nm.

1. Place 2g of zeolite in a 15 x 130mm screw-capped (Teflon) culture tube.
2. Add 10mL of chloroform, and shake.
3. Add 0.5mL of serum or plasma, and shake for 30 minutes on a mechanical shaker.
4. Filter through coarse fat-free filter paper.
5. Pipette 1 to 3mL portions of the filtered supernatant (depending on the amount of triglyceride present) into two 16 x 100mm test tubes.
6. Pipette 1 mL portions of the working standard into two tubes.
7. Evaporate all tubes to dryness under nitrogen gas.
8. To one set of standards and unknowns, add 0.5mL of alcoholic potassium hydroxide (saponified sample). To the other set, add 0.5mL of 95% ethanol (unsaponified sample).
9. Keep all tubes at 65^0C for 15 minutes.
10. Add 0.5mL of 0.2N sulfuric acid to each tube. Place uncapped tubes in a 100^0C water bath for 15 minutes to remove the alcohol.
11. Remove tubes from water bath, cap and allow to cool.
12. Add 0.1mL of periodate solution to each tube. Mix well.

13. After 10 minutes add 0.1 mL of sodium arsentie to stop oxidation. Mix well. A yellowish iodine colour appears and then vanishes in a few minutes.

14. Add 5.0 mL of chromotropic acid reagent. Mix well.

15. Heat the tubes in water bath at 100^0C for 30 minutes in the absence of excessive light.

16. Cool in dark to room temperature.

17. Read absorbance (A) at 570nm. The color remains stable for several hours.

Calculation:

Triglycerides (mg/L) =

$$\frac{A_{unknown\ (saponified)} - A_{unknown\ (unsaponified)}}{A_{standard\ (saponified)} - A_{standard\ (unsaponified)}} \times 500\text{mg/L} \times \frac{20}{V}$$

Where 500mg/L is the concentration of the triglyceride standard, 20 is the dilution factor (0.5mL sample to 10 mL chloroform) and V is the volume of filtered supernatant used in the assay. Multiply by 1.14 to convert milligrams per liter to micromoles per liter.

LDL & VLDL were calculated using Friedewald's Formula:

$$\text{LDL cholesterol (mg/dl)} = \text{Total cholesterol} - \frac{(\text{HDL} - \text{Cholesterol} + \text{triglyceride})}{5}$$

$$= \_\_\_\_\_ \text{mg\%}$$

$$\text{VLDL cholesterol (mg/dl)} = \frac{\text{Triglyceride}}{5}$$

$$= \_\_\_\_\_ \text{mg\%}$$

RESULTS

The present study comprises of 50 clinically diagnosed cases of acute myocardial infarction (25 cases of diabetes and 25 cases of hypertension) and 50 normal healthy controls. The age group ranges from 40 to 60 years. Statistical analysis was carried out by using students 't' test (unpaired)

The mean level of MDA in controls was 6.3 + 0.92 nmol/ml The value in cases (total) was 11.5 $\pm$ 0.72 nmol/ml and 11.2 $\pm$ 0.73 nmol/ml in diabetics with acute MI and 11.8 $\pm$ 0.61 in hypertensives. The level was significantly increased ($P < 0.0001$) in the cases (total) and the sub groups.

The value of SOD in controls was 940.4 $\pm$ 93.9 IU/g of Hb. The mean value of SOD was 450.0 $\pm$ 116.76 IU/g Hb in the total cases and 447.6 $\pm$ 99.19 (diabetics) and 453.2 $\pm$ 134.47 (hypertensives). The level of SOD was significantly decreased ($P<0.0001$) in all the total cases and the sub groups compared to controls.

The mean glutathione reducatase level was 9 $\pm$ 1.02 IU/g of Hb in controls and the level in cases (total) was 3.1 $\pm$ 0.79 IU/g of Hb and 2.9 $\pm$ 0.88 (Group I) and 3.3 $\pm$ 0.67 (Group II). The level of glutathione reducatase was decreased significantly ($P<0.0001$) in all the cases and sub groups compared to controls.

The mean catalase level in controls was 7.3 $\pm$ 0.70 IU/g of Hb and in total cases was 2.6 $\pm$ 0.53 IU/g of Hb and 2.6 $\pm$ 0.09 (Group I) and 2.7 $\pm$ 0.58 (Group II). The level of catalase was decreased significantly ($P<0.0001$) in all cases as well as the sub groups compared to controls.

The mean value of vitamin E in controls was 0.92 $\pm$ 0.10 mg/dl. The level of vitamin E in total cases was 0.54 $\pm$ 0.14 mg/dl and 0.52 $\pm$ 0.14 (Group I) and 0.56 $\pm$

0.15 (Group II). The level was significantly decreased (P<0.0001) in all the cases and sub groups compared to controls.

The mean vitamin C level in controls was 0.87 ± 0.09 mg/dl and in total cases was 0.51 ± 0.13 mg/dl and 0.47 ± 0.09 (Group I) and 0.55 ± 0.15 (Group II). The level of vitamin C was decreased significantly in all the cases and sub groups in comparison to controls.

The mean value of total cholesterol in controls was 170.6 ± 21.38 mg/dl and in total cases was 260 ± 28.12 mg/dl and 252.1 ± 31.72 (Group I) and 267.9 ± 21.83 (Group II). The value of total cholesterol was increased significantly in all the cases and the sub groups with acute myocardial infarction compared to controls.

The mean HDL-C level in controls was 39.6 ± 4.17 mg/dl and in cases (total) was 31.1 ± 0.83 mg/dl and 31.04 ± 0.86 (Group I) and 31.09 ± 0.82 (Group II). The level of HDL-C was decreased significantly (P<0.0001) in all the cases and sub groups compared to controls.

The mean LDL-C level in controls was 118.8 ± 27.43 mg/dl. The level of LDL –C in cases (total) was 151.3 ± 20.03 mg/dl and 158.56 ± 17.20 (Group I) and 144.04 ± 20.33 (Group II). The level of LDL –C was increased significantly (P<0.0001) in all the cases and sub groups compared to controls.

The mean value of VLDL-C in controls was 25.5 ± 5.51 mg/dl and in cases (total) was 34.2 ± 10.54 mg/dl and 34.09 ± 11.90 (Group I) and 33.88 ± 9.45 (Group II). The mean value of VLDL-C was significantly increased in all the cases and sub groups compared to controls (P<0.0001).

The mean triglyceride level was 118.4 ± 23.79 mg/dl in controls and in cases (total) was 157.7 ± 20.21 mg/dl and 159.04 ± 22.14 (Group I) and 155.56 ± 19.20

(Group II). The level of triglyceride was significantly raised (P<0.0001) in all the cases and sub groups compared to controls.

The mean value of blood glucose in the cases (n=25) was 151.16 ± 84.05 mg/dl. The level of blood sugar in controls was 87.90 ± 16.87 mg/dl. The value was increased significantly in cases compared to controls.

The mean value of systolic blood pressure in cases (n=25) was 147.04 ± 25.98 mm of Hg and in controls was 116.20 ± 10.39mm of Hg respectively. The value was increased significantly in the cases as compared to controls.

The mean value of diastolic blood pressure in the cases (n=25) was 95.12± 16.70mm of Hg and in the controls was 77.92 ± 10.11mm of Hg respectively. The level was significantly increased in cases compared to controls.

Table No: 4- Malondialdehyde (MDA), enzymatic and non- enzymatic antioxidants in total cases and controls

	MDA nmol/ml	**SOD IU/g of Hb**	**Glut red IU/g of Hb**	**Catalase IU/g of Hb**	**Vitamin E mg/dl**	**Vitamin C mg/dl**
n=50 **CONTROLS** Mean ± S.D	6.3 ± 0.92	940.4 ± 93.9	9 ± 1.02	7.3 ± 0.7	0.92 ± 0.1	0.87 ± 0.09
n=50 **CASES** Mean ± S.D	11.5 ± 0.72	450.8 ± 116.7	3.1 ± 0.79	2.6 ± 0.53	0.54 ± 0.14	0.51 ± 0.13
P. value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

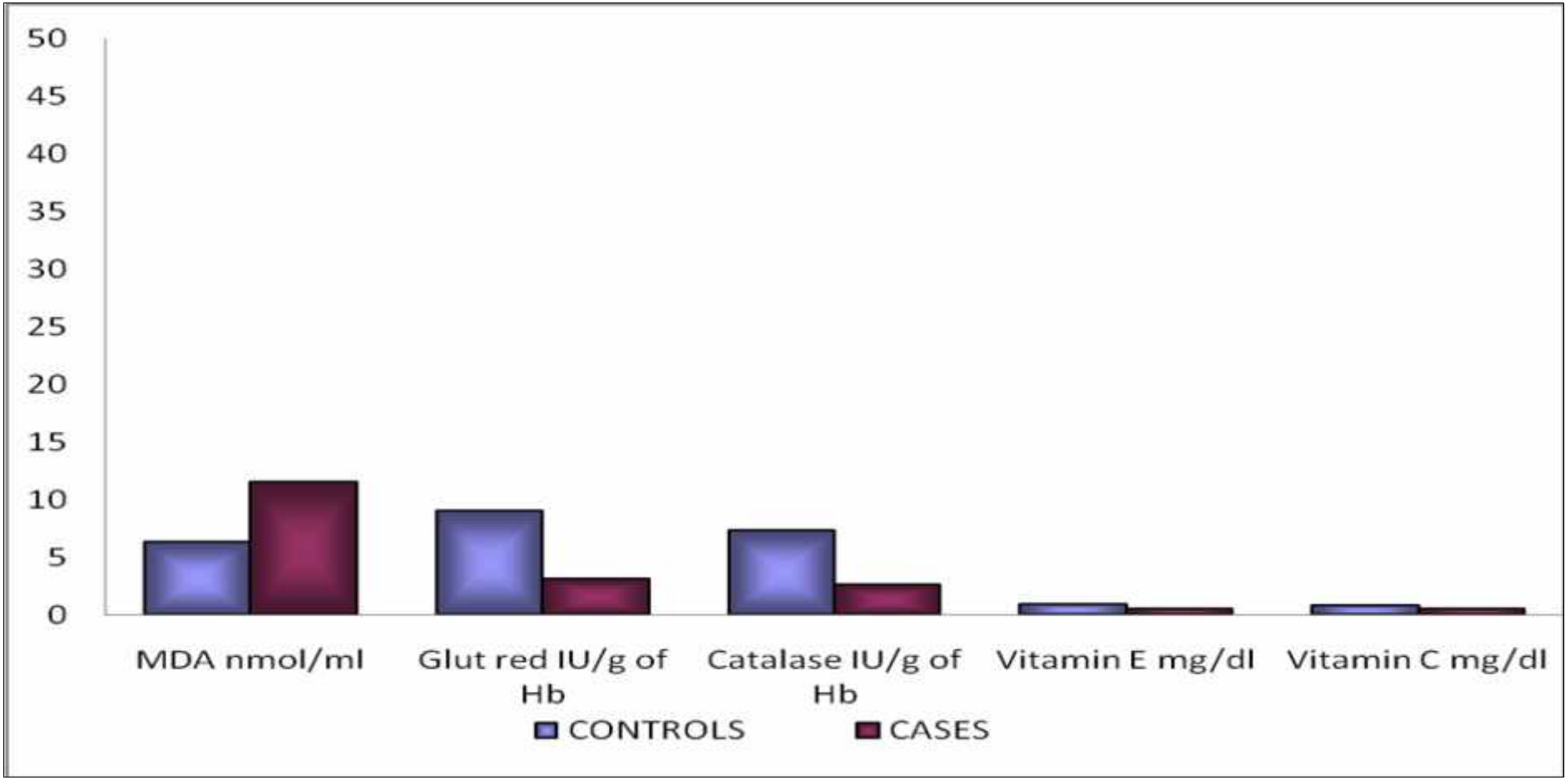
Table No: 5- Lipid Profile in total cases and controls

	T. Cholesterol (mg/dl)	**HDL (mg/dl)**	**LDL (mg/dl)**	**VLDL (mg/dl)**	**Triglyceride (mg/dl)**
n=50 **CONTROLS** Mean ± S.D	170.6 ± 21.38	39.6 ± 4.17	118.8 ± 27.4	25.5 ± 5.5	118.4 ± 23.79
n=50 **CASES** Mean ± S.D	260 ± 28.12	31.1 ± 0.83	151.3 ± 20.03	34.2 ± 10.54	157.7 ± 20.2
P. value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

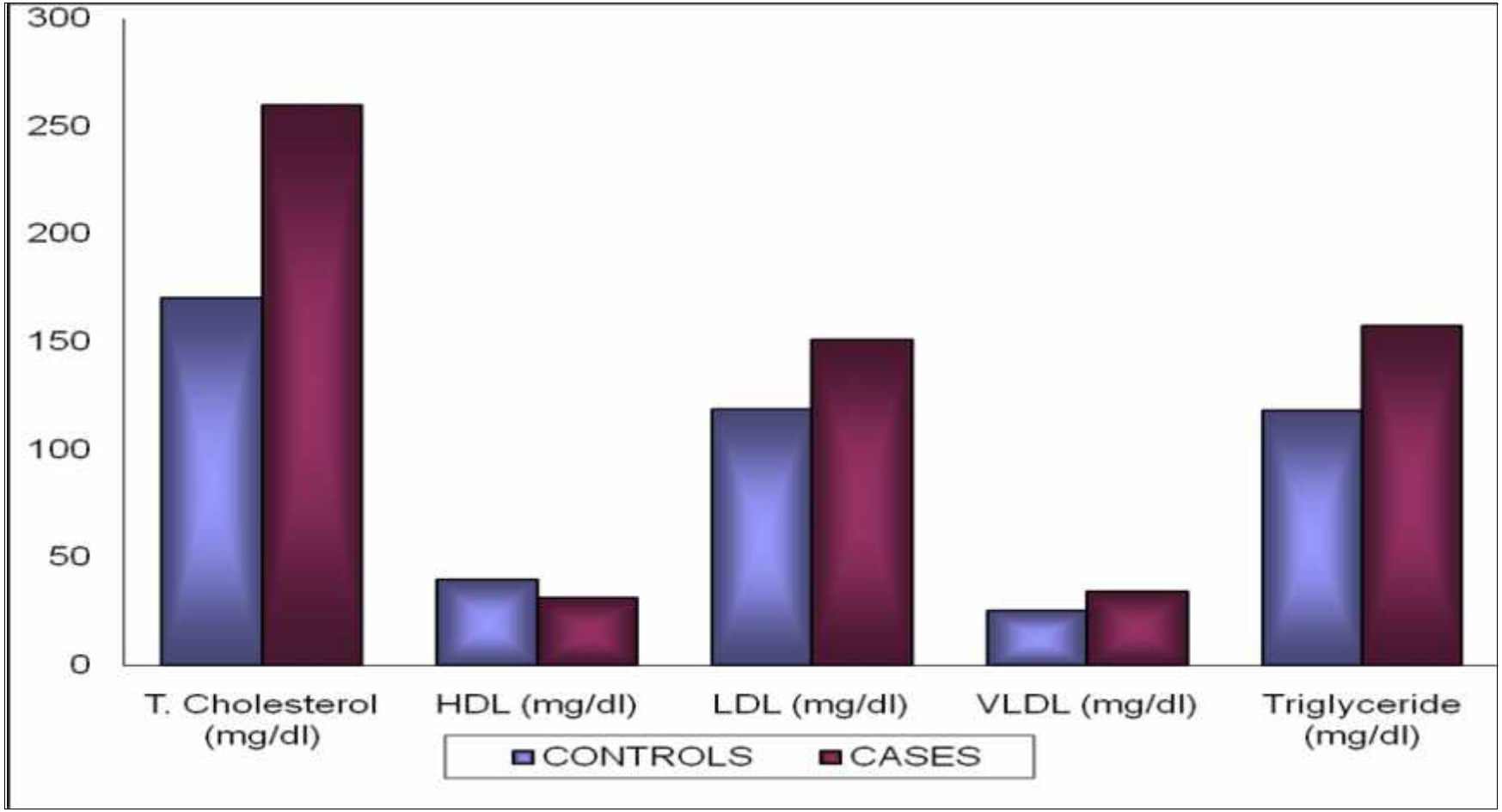
Table No: 6- Blood glucose and Blood pressure in cases and controls

	Blood glucose (mg/dl)	**Systolic B.P. (mm of Hg)**	**Diastolic B.P. (mm of Hg)**
n=50 **CONTROLS** Mean ± S.D	87.9 ± 16.87	116.2 ± 10.39	77.92 ± 10.11
n=25 **DIABETIC** Mean ± S.D	151.16 ± 84.05	118.8 ± 17.24	80.96 ± 14.0
n=25 **HYPERTENSIVE** Mean ± S.D	77.96 ± 22.36	147.04 ± 25.98	95.12 ± 16.7

Graph 1: Malondialdehyde (MDA), enzymatic and non- enzymatic antioxidants in total cases and controls



Graph 2: Lipid Profile in total cases and controls



Graph 3: Blood glucose and Blood pressure in cases and controls

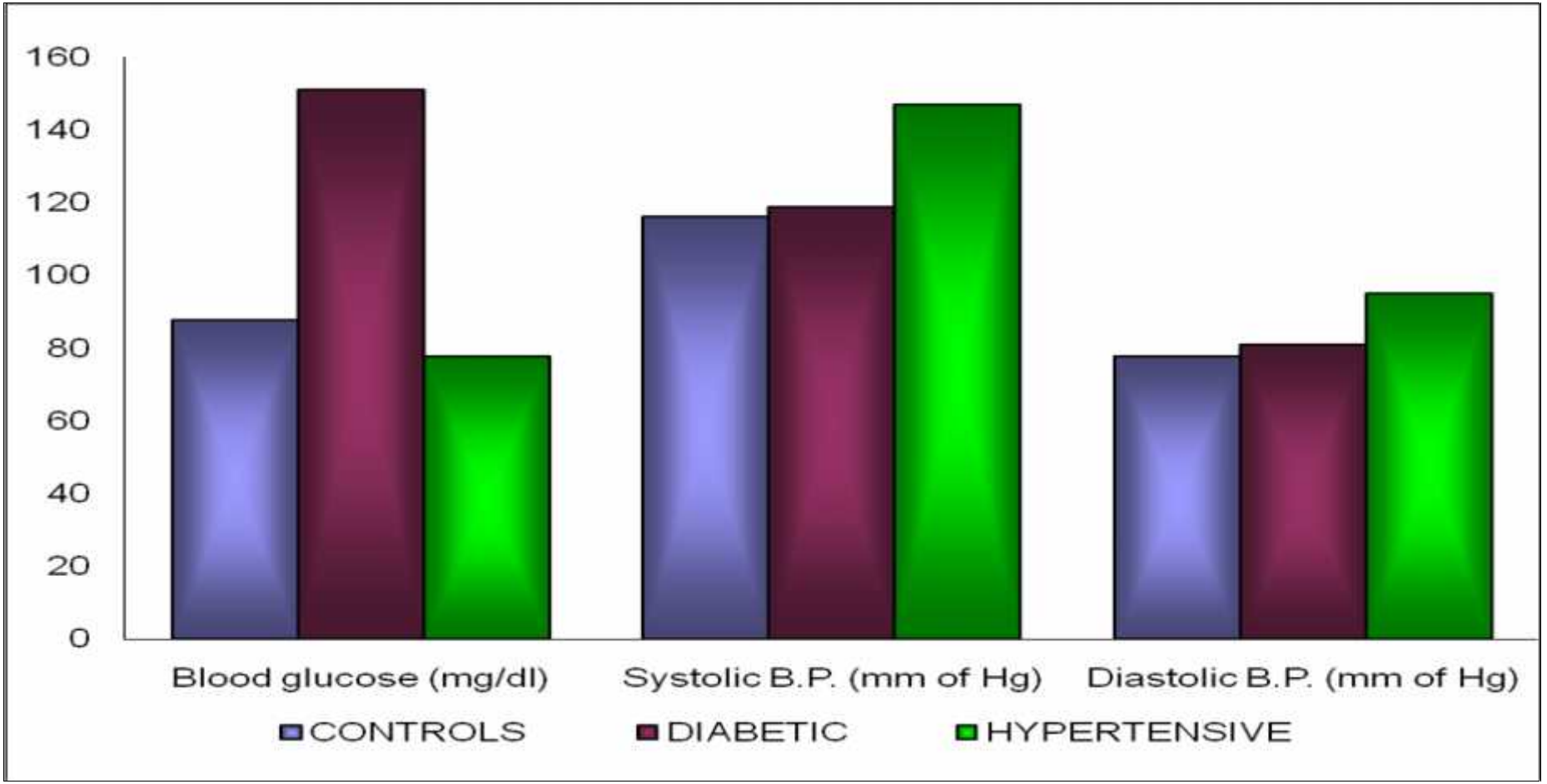


Table No : 7– MDA, enzymatic antioxidants, non-enzymatic antioxidants and lipid profile in diabetic and hypertensive with acute MI.

Parameters	Diabetic n=25	Hypertensive n=25	Controls	P. value
Malondialdehyde (nmol/ml)	11.2 ± 0.73	11.8 ± 0.61	6.3 ± 0.92	<0.005*
Superoxide dismutase (IU/g of Hb)	447.6 ± 99.19	458.2 ± 134.47	940.4 ± 93.9	<0.0001
Catalase (IU/g of Hb)	2.6 ± 0.09	2.7 ± 0.58	7.3 ± 0.7	<0.0001
Glutathione reductase (IU/g of Hb)	2.9 ± 0.88	3.3 ± 0.67	9 ± 1.02	<0.0001
Vitamin E (mg/dl)	0.52 ± 0.14	0.56 ± 0.15	0.92 ± 0.1	<0.0001
Vitamin C (mg/dl)	0.47 ± 0.09	0.55 ± 0.15	0.87 ± 0.09	<0.0001
Total Cholesterol (mg/dl)	252.1 ± 31.72	267.9 ± 21.83	170.6 ± 21.38	<0.0001
HDL (mg/dl)	31.04 ± 0.86	31.09 ± 0.82	39.6 ± 4.17	<0.0001
LDL (mg/dl)	158.56 ± 17.2	144.04 ± 20.33	118.8 ± 27.43	<0.0001
VLDL (mg/dl)	34.09 ± 11.90	33.88 ± 9.45	25.5 ± 5.51	<0.0001
Triglyceride (mg/dl)	159.04 ± 22.14	155.56 ± 19.20	118.4 ± 23.79	<0.0001

P<0.0001 is significant

DISCUSSION

Acute myocardial infarction is the major cause of mortality and morbidity worldwide. The root cause of AMI is mainly atherosclerosis initiated by oxidative stress. It is associated with various risk factors such as age, gender, diabetes mellitus, hypertension and smoking. An adverse lipid profile is an additional risk factor.

Involvement of oxygen free radicals in the pathophysiology of inflammation and ischemia in a number of organs and tissues has been reported in literature[74,75]. Evidence of OFR generation in patients with AMI has been observed by measuring the product of lipid peroxidation malondialdehyde. Antioxidant defense was assessed by measuring superoxide dismutase, catalase, glutathione reductase, vitamin E, vitamin C .Lipid profile was measured by estimating serum cholesterol, triglyceride, HDL-C, LDL-C,VLDL and were correlated with the controls and findings of other studies . Blood glucose and B.P. was measured in diabetic and hypertensives with acute M.I.

MALONDIALDEHYDE

In the present study mean level of MDA was increased significantly in cases compared to controls. Our findings are in accordance with the study of Neela Patil et al [76]. MDA is a decomposition product of lipid peroxidation of polyunsaturated fatty acids which is used as an index of oxidative damage. According to K Kaur et al[77] the high concentration of MDA in patients indicates increased membrane lipid peroxidation. Enhanced lipid peroxidation may occur as a result of imbalance between scavenging mechanisms and free radical generation process.

Our findings are contradictory to the findings of Kasp Segil et al [78] who showed significant decrease in MDA levels in acute M.I.

SUPEROXIDE DISMUTASE (SOD)

The level of SOD was significantly decreased in cases compared to controls (P < 0.0001). The findings of our study are in accordance with study of K Kaur et al [77]. Arun Kumar et al [79]and Gunasekera et al [80.]

According to K.Kaur et al [77], decrease in SOD activity may be attributed to hypoxia due to ischemia. There is an enhanced production of superoxide anions by ischaemic cells. Increased concentration of LDL causes uncoupling of endothelial nitric oxide synthase and consequently increased production of superoxide anions in the vessel wall. In patients with CAD, secretion of TNF- α and increased free radical load causes depression of extracellular SOD activity.

GLUTATHIONE REDUCTASE

The level of glutathione reductase was decreased significantly in cases (P<0.0001) compared to controls.

The findings of the present study are in accordance with the results of Simmi Kharb et al [81],R.Sivakanesan et al [82]. According to Simmi Kharb et al [81], OFR are generated particularly in the early stages of MI and glutathione reductase is involved in the reduction of hydrogen peroxide radicals, resulting in a decrease in glutathione reductase levels during that period.

Blaustein et al [83] have demonstrated that GSH is important in protecting the myocardium against OFR injury. Depressed levels may be associated with an enhanced protective mechanism to oxidative stress in AMI.

CATALASE

The level of catalase was decreased significantly in cases compared to controls.

Our findings are in accordance with Arun Kumar A et al [82] and Gunasekara et al [80].Free radical scavenging enzymes such as SOD and catalase are the first line of defense against oxidative injury, decomposing oxygen and hydrogen peroxide before interacting to form the more reactive hydroxyl radical. Decrease in the levels of catalase could be due to inactivation of the enzymes by cross-linking or due to exhaustion of the enzymes by increased peroxidation.

VITAMIN-E

The mean level of vitamin E was lowered significantly in cases compared to controls.

The findings of our study are in accordance with the study of Kumar A et al [82] and Palanisamy P et al [85], who have mentioned in their study that decreased level of vitamin E is because of its inability to combat increased oxidative stress and inflammation. Our results are contradictory to the findings of Reema Sood et al [84] who showed higher values of vitamin E level in patients of acute myocardial infarction.

VITAMIN C

The level of vitamin C was decreased significantly in cases compared to controls.

The findings of our study are in accordance with the study of Reema Sood et[83], Rudolph A et al [86] and Kristina N et al.

According to Reema Sood lowered levels of vitamin C is due to its enhanced consumption because of increased oxidant stress caused by free radicals which indicates asynchrony between oxidants and anti oxidants in AMI patients.

TOTAL CHOLESTEROL

The level of total cholesterol was significantly increased in cases compared to controls.

The findings of our study are in accordance with the findings of Ritu Sharma et al [88], Palanisamy P et al [84], R. Sivakanesan et al [89.]

According to Palanisamy P, changes in the concentration of plasma lipids including cholesterol are the complications frequently observed in patients with MI and certainly contribute to the development of vascular disease. Cholesterol has been singled out as the primary factor in the development of atherosclerosis.

HIGH DENSITY LIPOPROTEIN HDL-C

The level of HDL-C was decreased significantly ($P < 0.0001$) in the cases compared to controls.

The findings of the present study are in accordance with the study of Ritu Sharma et al [88], T Angeline et al [90] and William P et al [91]. HDL helps in the transport of cholesterol from the peripheral tissues to the liver where it is catabolised and excreted. Higher levels of HDL could be associated with less incidence of MI. According to them the strength of the inverse association between HDL & CHD is such that there is a twofold increased prevalence of CHD in subjects with low HDL.

According to Ritu Sharma et al, HDL levels were found to be significantly lowered, suggesting that loss of scavenger role of HDL could be the risk factor responsible for the causation of CHD in patients who had negative family history of coronary artery disease.

LOW DENSITY LIPOPROTEIN LDL-C

The level of LDL-C was raised significantly in cases compared to controls.

The findings of our study are in accordance with the study of K.Kumar et al [77], and Palanisamy P et al [85].

The findings are contradictory to the findings of P.K.Nigam et al[92] who showed no significant change in the value of LDL in patients of acute myocardial infarction. According to Palanisamy, the concentration of LDL correlates positively with the development of coronary heart disease.

VERY LOW DENSITY LIPOPROTEIN VLDL-C

The mean value of VLDL-C was significantly increased in cases as compared to controls ($P<0.0001$)

The findings of our study agree with the findings of Neela Patil et al [76] and Palanisamy P et al [85].

TRIGLYCERIDES

The level of triglyceride was significantly raised in the cases of acute myocardial infarction in comparison to controls.

The findings of our study are in accordance with the findings of Ritu Sharma et al [88], T. Angeline et al [90] and P.K.Nigam et al [92]. The mechanism of increase in triglycerides after MI maybe due to elevated flux of fatty acids and impaired removal of VLDL from the plasma.

BLOOD GLUCOSE

The level of blood glucose was increased significantly in cases (25) compared to controls.

The findings of our study are in accordance with the study of Neeta Patil et al[76] who showed that glucose and glycosylated hemoglobin levels were significantly high in diabetic AMI patients as compared to controls. In diabetes there is activation of hormone sensitive lipase leading to formation of more free fatty acids which are catabolised to produce acetyl coA which is channeled to cholesterol synthesis. Glycation of LDL also takes place in diabetes which has a prolonged half life . Oxidized LDL is taken up by non specific endocytosis further leading to atherosclerosis and consequently causing MI.

The findings of our research work agree with the study of William B et al[93], M.Ishihara et al [94] and Mario C. et al [95].

BLOOD PRESSURE

The mean value of systolic B.P. in cases (25) and controls was 147.0 ± 25.98 mm of Hg and 116.20 ±10.39mm of Hg respectively.

The mean value of diastolic B.P in cases and controls was 95.12 ± 16.70 mm of Hg and 77.9 ± 10.11 respectively.

There was significant increase in blood pressure in cases compared to controls. The findings of the present study are in accordance with the study of Mario C. et al [95], F.Gustafsson et al [96] and William B. et al [93]. According to F.Gustafsson et al, arterial hypertension is a moderate risk factor for morality after 65 years of age with acute myocardial infarction. Mechanical injury of the arterial wall due to increased blood pressure is the proposed mechanism for atherosclerosis in hypertensives with MI.

SUMMARY

Our study revealed that there was increased oxidative stress and decreased antioxidant defense in patients of acute myocardial infarction (Total, Group I and Group II) as evidenced by –

- Increased levels of malondialdehyde.
- Decreased levels of enzymatic antioxidants like superoxide dismutase, catalase and glutathione reductase.
- Decrease in the concentration of non- enzymatic antioxidants like vitamin E and Vitamin C.

There was altered lipid profile in the cases of acute myocardial infarction as evidenced by our study:

i) Increased total cholesterol

ii) Decreased HDL &

iii) Increased LDL, VLDL and triglyceride levels.

It is established fact that diabetic and hypertensive individuals are more prone to AMI as compared to healthy individuals.

CONCLUSION

In AMI there is an increased oxidative stress and decreased antioxidant defense (enzymatic and non- enzymatic) which is the root cause of atherosclerosis. Supplementation of natural antioxidants to the individual who are prone for AMI (i.e., diabetic and hypertensive) may prevent the occurrence of AMI to a certain extent.

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ANNEXURE – I

INFORMED CONSENT (MODEL OF THE CONSENT FORM IS ENCLOSED) DESCRIBING THE FOLLOWING.

a) Purpose of Study:

The purpose of the study is to know the status of oxidative stress and antioxidants in acute Myocardial Infarction.

There will be approximately 50 clinically diagnosed acute Myocardial Infarction patients and equal number of normal healthy volunteers participating in the study. It will be conducted under supervision of Dr. M. V. Kodliwadmath, Professor, Dept. of Biochemistry, JNMC, Belgaum and will be carried out by Dr.Vanishree, Postgraduate, Dept. of Biochemistry, JNMC, Belgaum for her MD dissertation to be submitted to KLE University, Belgaum.

b) Procedure and Treatment:

For patients of acute Myocardial Infarction:-

You will qualify for the study if you are diagnosed as acute Myocardial Infarction, in the age group of 40-60 years and willing to provide additional background and medical information.

Your venous blood sample (approximately 10ml will be collected for study under the aseptic precautionary measures using sterile disposable syringe. The required parameters will be estimated from the blood sample.

For Normal Individuals (Control Group):-

You will qualify for the study if you are in age group of 40-60 years, free from diseases, in good health and agree to provide additional background

and medical information. Your venous blood sample (approximately 10ml) will be collected for the study under aseptic precautionary measures using sterile disposable syringe.

The following parameters will be estimated from the blood of patients with acute Myocardial Infarction and control subjects.

- Malondialdehyde
- Superoxide dismutase
- Catalase
- Glutathione reductase
- Vitamin C
- Vitamin E
- Lipid Profile

c) Risks:

Since blood is drawn under aseptic precautionary measures by trained technicians there is no scope for any risk. Further, only small volume of blood is collected which will be spontaneously replenished in the body.

d) Benefits:

You can make use of levels of studied parameters if desired.

e) Financial incentive for participation:

You will not receive any payment for participating in this study.

f) Alternative:

If you decide not to participate in the study, the hospital will provide you usual standard care and treatment.

g) Authorization to publish results:

The study results will be published in the M.D. dissertation of Dr. Vanishree to be submitted to KLE University, Belgaum. Also the results may be published for scientific purposes and/or presented to scientific groups. However, you will not be identified.

h) Sponsors Policy:

Department of Biochemistry, JNMC, Belgaum is the sponsor of this study project which you are participating.

i) Institutional Policy:

The J. N. Medical College will provide within the limitations of laws of the state of Karnataka, facilities and medical attention to subjects who suffer any harm as a result of participating in this project. In the event you believe that you have suffered any harm as the result of your participation in this study you may contact the research guide Dr. M. V. Kodliwadmath or Dr. Vanishree.

j) Emergency Provision:

In the event of emergency you should telephone Cardiology Department.

k) Voluntary Participation:

Your participation in the study is voluntary. Your decision whether or not to participate will not affect care or treatment in the hospital. You are free to discontinue participation in this study at any time and for any reason. In case you need any further information regarding your rights as a study participant, you may please contact Dr.V.D. Patil, Principal, JNMC, Belgaum

and Chairman of the JNMC Institutional Ethics committee of Human Subjects Research.

l) Statement of Consent:

I volunteer and consent to participate in this study, I have read the consent or it has been read to me. The study has been fully explained to me and I may ask question at any time.

_____ _____

Signature Date

Name of Subject: _____

Sign of participants / guardian: Date:_____

Sign of researcher : _____ Date:_____

Sign of witness : _____ Date:_____

Name of witness : _____

ANNEXURE – II

QUESTIONNARE (PROFORMA) USED FOR COLLECTING THE DATA

"OXIDATIVE STRESS AND ANTIOXIDANT DEFENSE IN ACUTE MYOCARDIAL INFARCTION"- A CASE CONTROL STUDY.

Name : Sex:

Age : Occupation:

IP No. :

D.O.A. :

Address :

Presenting complaints:

1. Chest pain - onset: Site:
   Duration: Nature:
   Frequency: Radiation:

Aggravating & Relieving factors:

2. Sweating – Present / Absent
3. Dyspnoea
4. Palpitations
5. Giddiness / Syncope

Past History:

* H/O Ischaemic heart disease
* H/O Valvular heart disease
* H/O TIA / Stroke

Family History:

Of any ischaemic heart disease

Personal History:

* H/O smoking & alcohol consumption
* Drug intake
* Sleep
* Appetite
* Bowel & Bladder habits

General Physical Examination:

* Pallor
* Pedal edema
* Cyanosis
* Clubbing
* Pulse rate
* Respiratory rate
* Blood pressure
* Temperature

Systemic examination

Cardiovascular system

I Peripheral C.V.S.

* Pulse
* B. P.
* J. V. P.
* Other signs of C C F

II Central C.V.S.

* Inspection

* Palpation

* Percussion

* Auscultation

Respiratory system

Per abdomen

C. N. S. Examination

Investigations:

I	Blood	Hb%	RBS
		TC	FBS
		DC	PPBS
		ESR	Enzyme markers
II	Urine	Allbumin	
		Sugar	
		Microscopy	
III	Chest X – ray – PA view		
IV	ECG		

Final Diagnosis

ANNEXURE – III -MASTER CHART

CONTROL

Sl.No	CONTROLS	MDA nmol/ml	SOD IU/g of Hb	Glut red IU/g of Hb	Catalase IU/g of Hb	VitaminE mg/dl	Vitamin C mg/dl	T.chol mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl	Triglyc mg/dl
1	SSP	3.85	826.00	8.52	8.13	0.88	0.88	168.00	39.60	104.00	22.40	152.00
2	SGK	5.60	1011.20	8.34	9.10	0.96	0.70	172.00	36.00	152.00	19.20	98.80
3	VS	4.60	980.00	9.60	7.60	1.01	1.00	175.00	38.40	155.00	20.60	145.00
4	SJB	5.89	1111.10	10.04	8.00	0.88	0.92	186.00	46.10	128.00	24.20	138.00
5	PDC	7.75	925.00	9.10	9.52	0.79	0.84	163.00	44.80	104.00	25.60	172.00
6	SB	6.98	980.39	10.52	7.20	1.00	0.72	145.40	38.40	119.60	28.80	109.00
7	VSK	5.60	956.93	10.88	7.50	0.95	0.91	153.50	33.80	97.10	21.40	104.00
8	SMN	6.12	984.00	8.62	8.30	0.79	1.03	169.00	38.40	143.70	27.20	121.00
9	KYT	5.38	925.38	9.79	6.90	0.88	0.97	193.00	30.50	136.00	34.10	132.30
10	AG	6.15	757.57	8.76	7.70	0.77	0.73	200.00	31.70	81.40	25.60	147.80
11	DK	7.46	925.92	8.19	6.80	0.98	0.85	158.00	32.40	97.10	27.80	152.00
12	RSK	7.01	1000.00	9.79	6.50	0.88	0.88	185.80	41.10	90.20	19.20	156.00
13	HBD	5.06	956.93	10.37	8.20	0.95	0.88	140.00	42.60	103.60	24.20	155.00
14	DB	6.92	980.39	7.40	7.40	0.97	0.86	202.00	34.00	97.10	37.00	103.00
15	MI	6.12	925.92	8.84	6.90	0.82	0.83	169.70	36.90	126.20	31.00	128.00
16	PN	5.41	1041.60	8.19	7.00	1.13	0.82	153.50	39.00	116.80	34.10	144.00
17	MNS	6.98	888.96	7.19	6.80	0.81	0.95	170.00	40.20	113.80	25.60	64.00
18	CKB	7.30	1012.01	7.50	8.80	0.96	0.85	186.00	37.80	147.30	31.00	96.00
19	VSJ	6.98	980.39	9.78	6.98	0.98	0.88	200.00	38.40	136.00	28.30	152.00
20	CDK	5.60	1041.60	11.12	7.23	1.00	1.04	193.00	46.20	165.00	25.60	104.00
21	MSH	5.41	826.40	8.84	8.14	1.13	0.96	179.00	46.20	81.40	26.40	139.00
22	KK	6.21	1176.40	7.90	7.50	0.91	0.70	155.00	44.80	119.00	20.20	116.00
23	SBP	4.83	956.90	8.54	6.80	0.87	0.85	165.00	39.40	129.20	23.60	96.00
24	MP	5.06	959.08	8.19	6.50	0.95	1.10	138.00	37.80	141.00	24.40	103.70

Sl.No	CONTROLS	MDA nmol/ml	SOD IU/g of Hb	Glut red IU/g of Hb	Catalase IU/g of Hb	VitaminE mg/dl	Vitamin C mg/dl	T.chol mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl	Triglyc mg/dl
25	DM	6.15	1000.00	8.70	7.30	0.84	0.84	145.00	34.00	69.20	23.60	122.00
26	KVL	7.01	757.57	9.78	7.55	0.77	0.73	193.90	40.10	85.20	28.80	139.00
27	RJD	7.53	1136.36	9.60	6.93	1.02	0.85	217.50	41.60	105.00	21.40	142.00
28	SH	7.75	787.08	8.50	7.02	0.93	0.82	189.00	33.80	92.00	27.20	127.00
29	NPB	6.79	1075.26	8.70	6.38	0.82	0.97	178.00	36.50	114.20	28.80	118.00
30	SM	5.89	833.33	7.60	6.50	1.13	0.82	210.00	36.80	116.60	22.40	97.10
31	MJ	5.38	980.39	7.50	7.30	1.01	0.88	168.00	40.10	104.00	23.60	109.00
32	RKD	6.21	1041.66	8.20	6.90	0.84	0.93	186.00	34.00	69.20	20.80	129.00
33	RN	6.15	952.38	10.40	7.90	0.97	0.85	172.80	42.60	144.00	19.20	64.00
34	SL	6.79	984.53	9.80	6.70	0.82	0.87	140.00	38.60	170.30	22.40	103.70
35	DK	6.12	909.09	9.00	6.50	0.93	0.85	137.80	38.40	69.20	30.40	69.20
36	SKU	5.06	925.92	8.50	6.41	0.77	0.73	170.90	46.20	185.10	37.00	104.00
37	SYK	5.38	757.70	7.30	7.85	0.82	0.71	218.10	42.30	155.50	13.80	136.00
38	SI	6.08	823.05	8.90	7.50	0.92	0.85	178.00	43.00	128.00	20.70	130.00
39	ABG	7.32	984.60	10.10	6.80	1.15	0.98	155.00	42.50	112.00	34.10	110.00
40	MPY	7.45	854.30	7.90	6.90	0.87	0.76	153.50	40.10	104.00	25.60	128.00
41	RM	6.42	858.08	8.20	6.80	1.04	0.96	170.90	38.40	107.60	22.40	112.00
42	KS	6.15	888.07	8.80	7.10	0.93	0.84	154.30	46.10	119.00	27.80	96.00
43	VKP	7.23	941.18	9.30	7.60	0.81	0.92	147.00	38.40	81.40	20.30	98.50
44	SS	6.90	874.30	9.50	6.50	1.13	0.97	133.00	44.80	165.30	24.20	104.00
45	SIT	6.50	1014.08	10.40	7.40	0.95	0.88	172.40	46.10	143.00	21.40	106.00
46	VJP	7.32	896.15	11.00	7.20	0.84	0.91	180.90	42.00	132.80	37.00	118.00
47	RKP	5.99	815.35	9.90	6.90	0.83	0.89	185.00	36.00	119.40	13.80	101.30
48	YH	7.75	925.00	8.70	6.50	0.79	0.84	145.40	38.00	125.00	20.30	103.00
49	IJM	6.63	980.00	10.10	6.80	0.96	1.00	153.50	41.10	137.80	27.20	116.00
50	MVP	7.10	893.69	9.70	7.50	0.79	0.93	155.00	44.50	162.30	34.10	108.00

CASES (TOTAL)

Sl.No	CASES N=50	MDA nmol/ml	SOD IU/g of Hb	Glut red IU/g of Hb	Catalase IU/g of Hb	VitaminE mg/dl	Vitamin C mg/dl	T.chol mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl	Triglyc mg/dl
1	GVK	11.53	526.31	2.70	2.37	0.54	0.48	234.00	30.76	172.40	30.40	152.00
2	MSP	10.98	605.40	2.50	3.00	0.62	0.50	250.00	30.10	175.00	70.00	178.00
3	VB	11.00	592.50	2.45	2.59	0.34	0.45	240.00	31.30	183.00	65.00	210.00
4	ABK	11.57	488.60	1.95	2.90	0.58	0.37	210.00	30.40	179.00	62.00	190.00
5	MH	11.38	504.30	2.00	3.10	0.60	0.41	224.00	30.80	172.00	40.20	182.00
6	KK	11.82	526.30	2.10	2.50	0.43	0.45	226.20	30.70	117.00	27.20	136.00
7	PB	12.46	454.50	1.97	2.42	0.38	0.43	218.10	32.20	156.00	34.00	152.00
8	AJ	11.53	333.30	3.20	3.00	0.56	0.54	234.30	32.20	165.00	30.80	128.00
9	SS	10.98	263.15	2.74	2.83	0.35	0.51	210.00	30.76	168.00	24.00	144.00
10	KP	11.00	357.10	2.18	2.32	0.47	0.44	242.00	30.80	170.00	26.80	176.00
11	AS	9.90	416.60	3.27	3.10	0.50	0.47	185.80	31.50	165.00	28.80	170.00
12	BI	10.43	285.70	4.99	3.20	0.49	0.50	202.00	32.30	119.00	22.40	157.00
13	PD	11.53	534.80	2.80	3.20	0.40	0.50	236.80	30.30	123.00	40.50	163.00
14	RS	11.92	572.10	3.26	2.73	0.51	0.43	250.00	31.20	154.00	50.30	179.00
15	RK	11.18	478.30	2.50	2.60	0.45	0.39	275.00	30.80	172.00	43.80	164.00
16	DB	13.17	611.50	1.98	1.59	0.62	0.44	283.10	31.70	185.00	30.70	186.00
17	SJ	11.82	554.20	2.00	2.90	0.81	0.36	272.30	30.60	139.00	24.80	147.00
18	MVR	12.20	478.00	3.10	3.43	0.92	0.41	247.00	32.30	159.00	22.30	172.00
19	PD	12.46	503.00	2.43	2.57	0.37	0.51	239.10	31.90	164.00	29.80	159.00
20	SR	8.90	483.00	2.10	3.71	0.68	0.49	279.40	32.40	170.00	30.60	188.00
21	SC	10.90	614.40	4.13	2.19	0.36	0.53	285.30	30.40	168.00	34.10	147.00
22	MRP	11.50	598.30	3.88	3.43	0.57	0.39	290.00	31.80	139.00	40.10	183.00
23	NK	12.24	333.30	3.40	2.14	0.44	0.38	296.30	32.20	168.00	24.00	128.00
24	HV	12.20	277.70	2.54	2.04	0.32	0.35	257.80	30.76	174.80	30.40	136.00
25	SST	11.53	285.10	3.37	2.16	0.54	0.62	288.80	32.32	170.00	25.60	144.00

Sl.No	CASES N=50	MDA nmol/ml	SOD IU/g of Hb	Glut red IU/g of Hb	Catalase IU/g of Hb	VitaminE mg/dl	Vitamin C mg/dl	T.chol mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl	Triglyc mg/dl
26	BPBT	11.50	401.30	3.67	2.49	0.66	0.71	242.40	30.76	122.40	28.80	136.00
27	KDJ	11.35	476.10	3.40	2.14	0.55	0.63	280.00	28.80	144.00	24.00	149.00
28	AP	12.82	285.70	3.75	2.18	0.74	0.67	294.50	32.20	115.00	30.40	152.00
29	D.V	11.07	555.80	4.94	3.20	0.82	0.50	226.20	30.82	165.00	30.76	136.00
30	KK	11.45	416.00	3.37	1.97	0.63	0.43	278.30	32.42	156.00	32.20	158.00
31	RS	11.38	472.90	3.67	2.16	0.75	0.38	291.00	30.76	145.00	30.80	112.00
32	RM	11.00	285.70	3.56	2.14	0.51	0.61	287.30	31.20	168.00	24.00	172.00
33	RPP	12.25	263.10	3.75	2.10	0.48	0.58	276.40	30.80	117.00	28.80	176.00
34	S.A	12.10	416.50	2.77	2.37	0.37	0.47	285.80	30.14	150.40	22.40	160.00
35	SI	11.40	423.10	2.50	3.32	0.36	0.63	258.00	30.10	140.00	30.20	163.00
36	MM	11.92	239.80	3.80	2.00	0.49	0.58	262.00	31.30	127.00	29.60	178.00
37	RK	12.00	447.10	3.70	3.51	0.63	0.49	270.00	30.20	138.00	34.50	166.00
38	SB	11.41	502.70	3.10	2.14	0.52	0.39	285.10	30.00	144.00	40.10	135.00
39	KV	10.98	485.50	2.30	2.50	0.43	0.50	294.00	32.40	172.00	50.20	142.00
40	ARP	11.22	389.60	2.10	1.88	0.51	0.43	273.60	31.80	139.00	41.50	173.00
41	MSR	10.83	449.20	1.89	3.43	0.77	0.39	250.00	30.50	162.00	31.70	162.00
42	ADD	12.01	580.00	4.00	2.19	0.61	0.47	282.10	30.70	153.00	23.80	138.00
43	BSP	11.78	621.30	3.45	3.23	0.83	0.72	278.00	31.00	144.00	29.20	158.00
44	KST	11.00	581.90	3.67	1.88	0.66	0.85	300.00	32.00	135.00	37.90	163.00
45	JS	11.45	643.00	4.20	3.00	0.71	0.91	277.30	30.80	143.00	41.20	144.00
46	MRD	12.32	398.20	3.20	3.14	0.49	0.74	280.00	30.17	150.00	40.00	129.00
47	SPI	11.25	449.00	3.70	1.99	0.32	0.42	262.80	30.16	136.00	35.80	125.00
48	DT	11.10	532.60	4.13	3.00	0.38	0.57	253.00	30.11	125.00	37.20	144.00
49	KKS	12.32	229.30	3.75	2.98	0.47	0.64	248.00	30.30	130.00	36.80	167.00
50	LJ	10.99	298.40	2.90	2.75	0.54	0.72	288.10	31.23	117.00	29.00	175.00

GROUP - I

Sl.No	Diabetic	MDA nmol/ml	SOD IU/g of Hb	Glut red IU/g of Hb	Ctalase IU/g of Hb	Vitamin E mg/dl	Vitamin C mg/dl	T-cholest mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl	Triglyc mg/dl
1	GVK	11.53	526.31	2.70	2.37	0.54	0.48	234.00	30.76	172.40	30.40	152.00
2	RS	11.92	572.10	3.26	2.73	0.51	0.43	250.00	31.20	154.00	50.30	179.00
3	RK	11.18	478.30	2.50	2.60	0.45	0.39	275.00	30.80	172.00	43.80	164.00
4	STP	10.43	285.70	4.99	3.20	0.49	0.50	202.00	32.30	119.00	22.40	157.00
5	HV	12.20	277.70	2.54	2.04	0.32	0.35	257.80	30.76	174.80	30.40	136.00
6	VB	11.00	592.50	2.45	2.59	0.34	0.45	240.00	31.30	183.00	65.00	210.00
7	ABK	11.57	488.60	1.95	2.90	0.58	0.37	210.00	30.40	179.00	62.00	190.00
8	MH	11.38	504.30	2.00	3.10	0.60	0.41	224.00	30.80	172.00	40.20	182.00
9	PB	12.46	454.50	1.97	2.42	0.38	0.43	218.10	32.20	156.00	34.00	152.00
10	KP	11.00	357.10	2.18	2.32	0.47	0.44	242.00	30.80	170.00	26.80	176.00
11	AS	9.90	416.60	3.27	3.10	0.50	0.47	185.80	31.50	165.00	28.80	170.00
12	SJ	11.82	554.20	2.00	2.90	0.81	0.36	272.30	30.60	139.00	24.80	147.00
13	SP	8.90	483.00	2.10	3.71	0.68	0.49	279.40	32.40	170.00	30.60	188.00
14	RS	11.38	472.90	3.67	2.16	0.75	0.38	291.00	30.76	145.00	30.80	112.00
15	SI	11.40	423.10	2.50	3.32	0.36	0.63	258.00	30.10	140.00	30.20	163.00
16	SS	10.98	263.15	2.74	2.83	0.35	0.51	210.00	30.76	168.00	24.00	144.00
17	SCK	10.90	614.40	4.13	2.19	0.36	0.53	285.30	30.40	168.00	24.00	128.00
18	BPT	11.50	401.30	3.67	2.49	0.66	0.71	242.40	30.76	122.40	28.80	136.00
19	KDJ	11.35	476.10	3.40	2.14	0.55	0.63	280.00	28.80	144.00	24.00	149.00
20	DV	11.07	555.80	4.94	3.20	0.82	0.50	226.20	30.82	165.00	30.76	136.00
21	KK	11.45	416.00	3.37	1.97	0.63	0.43	278.30	32.42	156.00	32.20	158.00
22	RM	11.00	285.70	3.56	2.14	0.51	0.61	287.80	31.20	168.00	24.00	172.00
23	SA	12.10	416.50	2.77	2.37	0.37	0.47	285.80	30.14	150.40	22.40	160.00
24	KV	10.98	485.50	2.30	2.50	0.43	0.50	294.00	32.40	172.00	50.20	142.00
25	DRP	11.22	389.60	2.10	1.88	0.51	0.43	273.60	31.80	139.00	41.50	173.00

GROUP – II

Sl.No	Hypertensive	MDA nmol/ml	SOD IU/g of Hb	Glut red IU/g of Hb	Ctalase IU/g of Hb	Vitamin E mg/dl	Vitamin C mg/dl	T-cholest mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl	Triglyc mg/dl
1	MRP	11.50	598.30	3.88	3.43	0.57	0.39	290.00	31.80	139.00	40.10	183.00
2	PB	12.30	239.80	3.80	2.00	0.49	0.58	262.00	31.30	127.00	29.60	178.00
3	DB	13.17	611.50	1.98	1.59	0.62	0.44	283.10	31.70	185.00	30.70	186.00
4	MSP	10.98	605.40	2.50	3.00	0.62	0.50	250.00	30.10	175.00	70.00	178.00
5	PD	11.53	534.80	2.80	3.20	0.40	0.50	236.80	30.30	123.00	40.50	163.00
6	KK	11.82	526.30	2.10	2.50	0.43	0.45	226.20	30.70	117.00	27.20	136.00
7	AJ	11.53	333.30	3.20	3.00	0.56	0.54	234.30	32.20	165.00	30.80	128.00
8	MVR	12.20	478.00	3.10	3.43	0.92	0.41	247.00	32.30	159.00	22.30	172.00
9	PD	12.46	503.00	2.43	2.57	0.37	0.51	239.10	31.90	164.00	29.80	159.00
10	NK	12.24	333.30	3.40	2.14	0.44	0.38	296.30	32.20	168.00	24.00	128.00
11	SST	11.53	285.10	3.37	2.16	0.54	0.62	288.80	32.32	170.00	25.60	144.00
12	AP	12.82	285.70	3.75	2.18	0.74	0.67	294.50	32.20	115.00	30.40	152.00
13	RPP	12.25	263.10	3.75	2.10	0.48	0.58	276.40	30.80	117.00	28.80	176.00
14	SB	11.41	502.70	3.10	2.14	0.52	0.39	285.10	30.00	144.00	40.10	135.00
15	MSR	10.83	449.20	1.89	3.43	0.77	0.39	250.00	30.50	162.00	31.70	162.00
16	ADD	12.01	580.00	4.00	2.19	0.61	0.47	282.10	30.70	153.00	23.80	138.00
17	BSP	11.79	621.30	3.45	3.23	0.83	0.72	278.00	31.00	144.00	29.20	158.00
18	KST	11.00	581.90	3.67	1.88	0.66	0.85	300.00	32.00	135.00	37.90	163.00
19	JS	11.45	643.00	4.20	3.00	0.71	0.91	277.30	30.80	143.00	41.20	144.00
20	MRD	12.32	398.20	3.20	3.14	0.49	0.74	280.00	30.17	150.00	40.00	129.00
21	SPI	11.25	449.00	3.70	1.99	0.32	0.42	262.80	30.60	136.00	35.80	125.00
22	DT	11.10	532.60	4.13	3.00	0.38	0.57	253.00	30.11	125.00	37.20	144.00
23	KKS	12.32	229.30	3.75	2.98	0.47	0.64	248.00	30.00	130.00	36.80	167.00
24	LT	10.99	298.40	2.90	2.75	0.54	0.72	288.10	31.23	117.00	29.00	175.00
25	RK	12.00	447.10	3.70	3.51	0.63	0.49	270.00	30.20	138.00	34.50	166.00