ISCHEMIA MODIFIED ALBUMIN – AN EARLY MARKER OF MYOCARDIAL ISCHEMIA

Dissertation
M.D. (BRANCH XIII)
BIOCHEMISTRY

GOVT. STANLEY MEDICAL COLLEGE AND HOSPITAL,

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY,

CHENNAI – INDIA.

MARCH 2010.

CERTIFICATE

Certified that, following is the bonafide work done by **Dr. R. PANIMATHI., M.D.**, (Biochemistry), Stanley Medical College, Chennai, on the title **ISCHEMIA MODIFIED ALBUMIN - AN EARLY MARKER OF MYOCARDIAL ISCHEMIA,** as part of her dissertation during the year **2007-2010**.

DEAN **PROFESSOR AND HEAD OF THE DEPARTMENT**

DECLARATION

I, **Dr. R. PANIMATHI**, solemnly declare that the dissertation titled **ISCHEMIA MODIFIED ALBUMIN – AN EARLY MARKER OF MIOCARDIAL ISCHEMIA** is a bonafide work done by me at Government Stanley Medical College and Hospital, Chennai during the period from March 2009 to September 2009 under the guidance of **Dr. R. LALITHA**., M.D., Prof. and HOD, Department of Biochemistry, Government Stanley Medical College and Hospital, Chennai.

This dissertation is submitted to the Tamil Nadu Dr. MGR Medical University towards partial fulfillment of requirement for the award of **M.D** Degree in Biochemistry (Branch XIII).

Dr. R. PANIMATHI

ACKNOWLEDGEMENT

I express my sincere thanks to Professor Dr. Priya, M.S., Dean, Govt. Stanley Medical College and Hospitals, Chennai and the former Dean, Prof. Dr. Mohanasundaram., M.D., for granting me permission to utilize the facilities of this institution for my study.

I pay my sincere gratitude, respect and dedicate my work to my Prof Dr. R. Lalitha., M.D., Head of the Department of Biochemistry, Govt. Stanley Medical College.

I am extremely thankful to Prof. Dr. Subramaniam., M.D.,D.M., Head of the Department of Cardiology, Govt. Stanley Medical College and Hospitals for granting me permission to carry out my project in his department.

I express my sincere thanks to my former professor Dr.P.Jayanthi.,M.D, for initiating this thesis and Dr.Rengaramani.,M.D, for her guidance and, present Professors Dr.V.Amuthavalli., M.D., and Dr.K.Pramila., M.D., for their constant assistance in my work.

I also thank Assistant professors Dr. R. Shanthi., M.D., Dr.K.Chelladurai., M.D, Dr.R.Sureka., M.D., and all my colleagues for their help in my study.

I thank all the technical staffs for their cooperation and volunteers for their contribution to the study.

CONTENTS

ABBREVIATIONS

IHD	–	Ischemic Heart disease.
CHD	–	Coronary Heart disease
CVD	–	Cardio Vascular disease
IMA	–	Ischemia Modified Albumin
CK-MB	–	Creatine Kinase – MB isoform
LDH	–	Lactate dehydrogenase
AST	–	Aspartate transaminase
ECG	–	Electro Cardiogram
WHO	–	World Health Organization
MI	–	Myocardial infarction
TC	–	Total cholesterol
TAG	–	Tri acyl glycerol
HDL	–	High density lipoprotein
VLDL	–	Very low density lipoprotein
LDL	–	Low density lipoprotein.

INTRODUCTION

Myocardial Ischemia occurs when there is an inadequate supply of blood to the myocardium resulting in an imbalance between myocardial oxygen supply and demand.

The most common cause of Myocardial ischemia is an atherosclerotic disease of an epicardial coronary artery leading to a regional reduction in myocardial blood flow, followed by spasm, arterial thrombi, and rarely coronary emboli.

Episodes of defective perfusion result in decreased myocardial tissue oxygen tension, and cause transient disturbance of mechanical, biochemical and electrical functions of the myocardium. More severe the block, more the damage to the myocardium, also in relation to the duration of oxygen supply.

Early diagnosis of Myocardial Infarction at this stage itself can prevent morbidity and mortality. Though we have various myocardial proteins in the serum like CK-MB, LDH, AST and Troponins I and T for diagnosing Acute Myocardial Infarction, along with ECG, we should be able to identify myocardial ischemia

before it progresses to irreversible myocardial cell damage. These markers are not elevated in the absence of necrosis when measured in the first 2 to 6 hours following an ischemic event.

The present study on ischemia modified albumin has been found to be very useful for the detection of myocardial ischemia within 6 hours of onset of chest pain.

Ischemia Modified Albumin (IMA) is measured by Albumin Cobalt Binding assay. It is based on the reduced binding affinity of human serum albumin for metal ions like Cobalt attributed to the free radical damage to the N - terminal region of albumin in patients with myocardial ischemia [1].

The values are correlated with CK-MB and lipid profile.

AIM OF THE STUDY

Aim of the study is to measure the Ischemia Modified Albumin by Albumin Cobalt Binding assay in patients, within 6 hours of onset of chest pain.

Objective :-

(1) . To correlate the IMA values with CK-MB

(2) . Correlation of other markers of atherosclerosis like

(a). Total cholesterol

(b). Triacylglycerol

(c). High density lipoprotein

(d). Low density lipoprotein

(e). Very low density lipoprotein with IMA

(3). To prove the use of IMA as an early marker of myocardial ischemia.

REVIEW OF LITERATURE

Ischemic Heart Disease (IHD) [CHD – Coronary Heart Disease - synonym] has been defined as impairment of heart function due to inadequate blood flow to the heart muscles, compared to its needs, caused by obstructive changes in the Coronary circulation to heart [2].

Global burden of CHD depend on its mortality risk, decrease in life expectancy, increase in age specific death rates and proportion of death.

EPIDEMIOLOGY :-

Coronary Heart Disease is the cause of 25 – 30% of death in most industrialized countries[3]. At the beginning of the twenty first century, cardio vascular diseases (CVD) accounted for nearly half of all the deaths in the developed world and twenty five percent in the developing world [4].

WHO predicted that by 2030, 25 million deaths worldwide will occur due to CVD annually [5].

RISK FACTORS FOR CORONARY HEART DISEASE :-

Many prospective studies like Framingham Study established the Coronary Heart Disease Risk factors (i.e.) factors that make the occurrence of the disease more probable [6].

NON – MODIFIABLE RISK FACTORS	MODIFIABLE RISK FACTORS
Age	High Blood Pressure
Sex	Cigarette smoking
Family History	Diabetes
Genetic Factors	Obesity
Type A personality	Elevated Serum Cholesterol
	Sedentary Life Style
	Alcohol

NON MODIFIABLE RISK FACTORS

AGE :-

Age has a dominant influence. The peak period is between 40 to 60 years but also occurs earlier when other risk factors co exist [6].

SEX :-

Males are more affected by IHD than females. Clinical IHD in pre menopausal women is very low due to estrogenic effects.

However the incidence increases when oral contraceptive pills are used, which may precipitate hypertension [7].

After menopause, the rate of coronary events accelerates and equalizes to the levels observed in men [8].

FAMILY HISTORY :-

A family history of CHD (i.e.) less than 45 years in First degree male relatives and less than 55 years in female First degree relatives is known to increase the risk of premature death [9].

GENETIC FACTORS :-

The familial predisposition to atherosclerosis and Coronary Heart Disease is polygenic. They are probably the determinants of genetic derangements of lipoprotein metabolism [9] and also related to the clustering of other risk factors.

PERSONALITY :-

Type A behaviour doubles the risk of Coronary Heart Disease in otherwise healthy men. It was described as an important risk factor in Coronary Disease in 1950's by Cardiologist Meyer Friedman and his coworkers.

Type A personality includes a set of characteristics of being impatient, time conscious, insecure about one's status, highly competitive, hostile and aggressive and incapable of relaxation [10].

MODIFIABLE RISK FACTORS :-

HIGH BLOOD PRESSURE :-

Hypertension accelerates the atherosclerotic process especially if hyper lipidemia is also present. Both the systolic and diastolic components are risk factors. There is a fivefold increase of CHD when the blood pressure exceeds 140/90 mm of Hg [11].

CIGARETTE SMOKING :-

The degree of risk of developing CHD is related to the number of cigarettes smoked per day. Smoking accelerates atherosclerosis and promotes acute ischemic events [8,12].

The mechanisms are :-

a. Carbon monoxide induced atherogenesis and relative hypoxemia.
b. Nicotine stimulation of adrenergic drive increasing both heart rate and myocardial oxygen demand.
c. Endothelial injury and dysfunction due to nitric oxide release.
d. Enhanced coagulability and induced chronic inflammatory state.
e. Development of atherogenic lipid profile (higher levels of low density lipoprotein, more oxidized low density lipoprotein, and lower levels of High density lipoprotein.

OBESITY :-

Greater the weight gain greater is the risk of hyper tension, CHD and insulin resistance diabetes mellitus.

Body mass index of $\geq$ 30 Kg/m^2 is considered as “obesity” and it plays a major role in atherosclerotic progression [13, 14].

ELEVATED SERUM CHOLESTEROL:-

A triangular relationship exists between habitual diet, blood cholesterol, lipoprotein levels and Coronary Heart Disease [14].

Presence of hyper cholesterolemia is sufficient to initiate the disease process by inducing an endothelial injury in the coronary arteries. Serum cholesterol concentration associated with low density lipoprotein which serves as a vehicle for the delivery of cholesterol to peripheral tissues play a major role in precipitating CHD [15].

HDL cholesterol acts as 'Good Cholesterol' against the development of CHD by mobilizing the cholesterol from the developing atheroma and transport to liver for excretion [16, 17] .

DIABETES:-

CHD is 2-3 times higher in diabetics than in non diabetics. It is responsible for 30-50% of death in diabetics over the age of 40 years in industrialized countries [18, 19] .

ALCOHOL:-

High alcohol intake defined as 75g or more per day becomes an independent risk factor for CHD [20].

PHYSICAL ACTIVITY:-

Sedentary life style with reduced physical activity leads to an early development of CHD.

Regular physical exercises increase the concentration of HDL and decrease both body weight and blood pressure which are beneficial to cardiovascular health [5].

ASYMPTOMATIC VERSUS SYMPTOMATIC IHD:-

In asymptomatic patients only the exercise stress tests may show evidence of myocardial ischemia.

In symptomatic phase, the patient may exhibit a stable or progressive course characterized by chest discomfort (Anginal chest pain) [9].

CLINICAL TYPES OF IHD:-

Patients with IHD fall into two large groups.

1. Patients with chronic coronary artery disease.
2. Acute coronary syndromes.

CHRONIC CORONARY ARTERY DISEASE:-

These patients commonly present with ANGINA PECTORIS where the ischemia causes pain but is insufficient to lead to death of myocardium.

Angina may be of different types.

STABLE ANGINA :-

When the episodes are provoked by exertion, emotion and relieved by rest due to fixed atheromatous stenosis of one or more coronary arteries [21].

ECG may be normal but sometimes shows ST depression, especially after exercise, usually on a treadmill.

OTHER ANGINAL TYPES :-

ANGINA DECUBITUS	-	Occurs in recumbent positions.
NOCTURNAL ANGINA	-	During sleep
STABLE EXERTIONAL ANGINA	-	Angina occurring predictably at a certain level of exertion.
UNFAMILIAR TASKS	-	Angina may also be precipitated by heavy meal, exposure to cold etc.

PRINZMETAL'S VARIANT ANGINA:-

Prinzmetal's variant angina is described as a syndrome of ischemic pain that occurs at rest but not usually with exertion and is associated with transient ST segment elevation. This is due to focal spasm of an epicardial coronary artery leading to severe myocardial ischemia [22].

Exact cause is not known, but may be related to vasoconstrictor mitogens, leukotrienes and serotonin or due to migraine; Reynaud's phenomenon or aspirin induced asthma.

ACUTE CORONARY SYNDROME (ACS) :-

Acute Coronary Syndrome comprises of a spectrum of disease that encompasses ischemia with minimal myocardial damage (i.e.) unstable angina and Myocardial Infarction. This infarction may be

1. STEMI – (ST elevation myocardial infarction)
2. NSTEMI – (Non ST elevation myocardial infarction) [23]

Several scenarios fulfill this broad definition of the syndrome, including a single episode of prolonged rest angina (at least 20 minutes), new onset of angina (less than 2 months) and acceleration of angina (an angina pattern that becomes more frequent, longer in duration, or lower in threshold) [24].

UNSTABLE ANGINA (PRE INFARCTION ANGINA):-

Unstable angina is characterized by new onset or rapidly worsening angina (crescendo angina), angina on minimal exertion or angina at rest as a result of dynamic obstruction of a coronary artery due to plaque rupture with super imposed thrombosis and spasm [25].

MYOCARDIAL INFARCTION:-

Myocardial Infarction usually results from a marked impairment in blood flow produced by a coronary occlusion resulting from a severe atherosclerotic plaque or from a thrombosis caused by platelet aggregation at the site of an unstable plaque that ruptured less frequently; embolic phenomena or vasospasm also precipitates MI [26].

ST - ELEVATION MYOCARDIAL ISCHEMIA (STEMI):-

Patient presents with new evidence of ST segment elevation on ECG and labeled as ST segment elevation Myocardial Infarction.

STEMI occurs when a coronary artery thrombosis develops rapidly at a site of vascular Injury previously affected by atherosclerosis.

NON ST - ELEVATION MYOCARDIAL ISCHEMIA (NSTEMI) :-

Those without ST segment elevation but with evidence of myonecrosis are deemed to have a non ST segment elevation myocardial infarction [27].

ETIOLOGY OF ISCHEMIC HEART DISEASE:-

Causes are based on

1) Reduction in blood flow
2) Increase in oxygen demand [1].

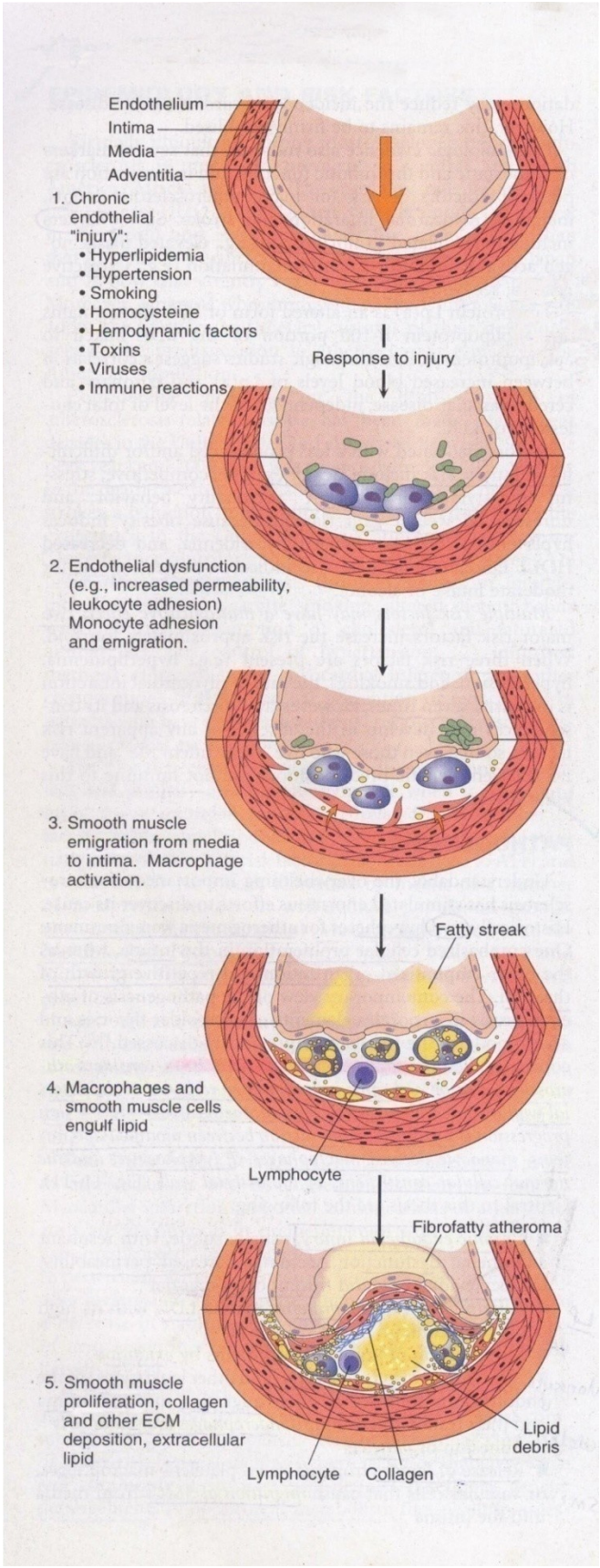
REDUCTION IN BLOOD FLOW:-

a) Atherosclerosis reduces the lumen of the coronary arteries and limits appropriate increase in perfusion, when the demand for flow is augmented as occurring during exertion or excitement.
b) Coronary blood flow can be limited by spasm as in prinzmetals variant angina.
c) Arterial Thrombi
d) Coronary emboli
e) Ostial narrowing due to arteritis.
f) Other causes [4].

INCREASED MYOCARDIAL OXYGEN DEMAND IN:-

1) Severe left ventricular hypertrophy due to aortic stenosis.
2) A reduction in oxygen carrying capacity of blood as in presence of carboxy haemoglobin.

PATHOGENISIS OF ATHEROSCLEROSIS



[From Kumar. V, Cotran. R, Robbins. S, Robbins basic pathology, edi-7, Philadelphia, 2003, Saunders, p522]

PATHOGENISIS OF ATHEROSCLEROSIS:-

Atherosclerosis is a complex disease that involves lipoprotein influx and modification, increased prooxidant stress and inflammatory angiogenic and fibro proliferative responses intermingled with extra cellular matrix and smooth muscle cell proliferation resulting in the formation of atherosclerotic plaque [28].

The overwhelming clinical importance of atherosclerosis is attributed to its epidemiologic data which reflects death caused by IHD.

RESPONSE TO INJURY:-

The concept of response to injury hypothesis considers atherosclerosis as a chronic inflammatory response of the arterial wall to the injured endothelium [29, 6].

COURSE OF ATHEROSCLEROSIS:-

Chronic endothelial injury due to toxins from smoking, hypertension, hyperlipedemia, hemodynamic factors (i.e.) at branch points lead to endothelial dysfunction causing increased

permeability and accumulation of lipoproteins in the intima of the vessel wall [30, 31].

Oxidative modification of these accumulated plasma lipoproteins (LDL) associated with proteoglycans occur within the intima of the arteries, forming fatty streak [32].

When it contacts blood, such modified lipoprotein trigger a local inflammatory response signaling the migration of monocyte derived macrophages and lymphocytes converting them to foam cells, mediated by cytokines like IL-I(Interleukin-I) and TNF (Tumor necrosis factor) [33, 34].

VCAM-I (Vascular cell adhesion molecule) in particular binds monocytes and T cells. Activated leukocytes and vascular wall cells release growth factors [35,36] (Growth factors like platelet derived growth factor, fibro blast growth factor, transferring growth factor) that promote SMC(Smooth muscle cell) proliferation and Extra cellular matrix synthesis(ECM) converting it into a mature atheroma and contribute to the progressive growth of atheromatous plaques, forming lesions [37,38].

ACUTE CHANGES OF PLAQUE:-

The initiating events that disrupts a plaque are

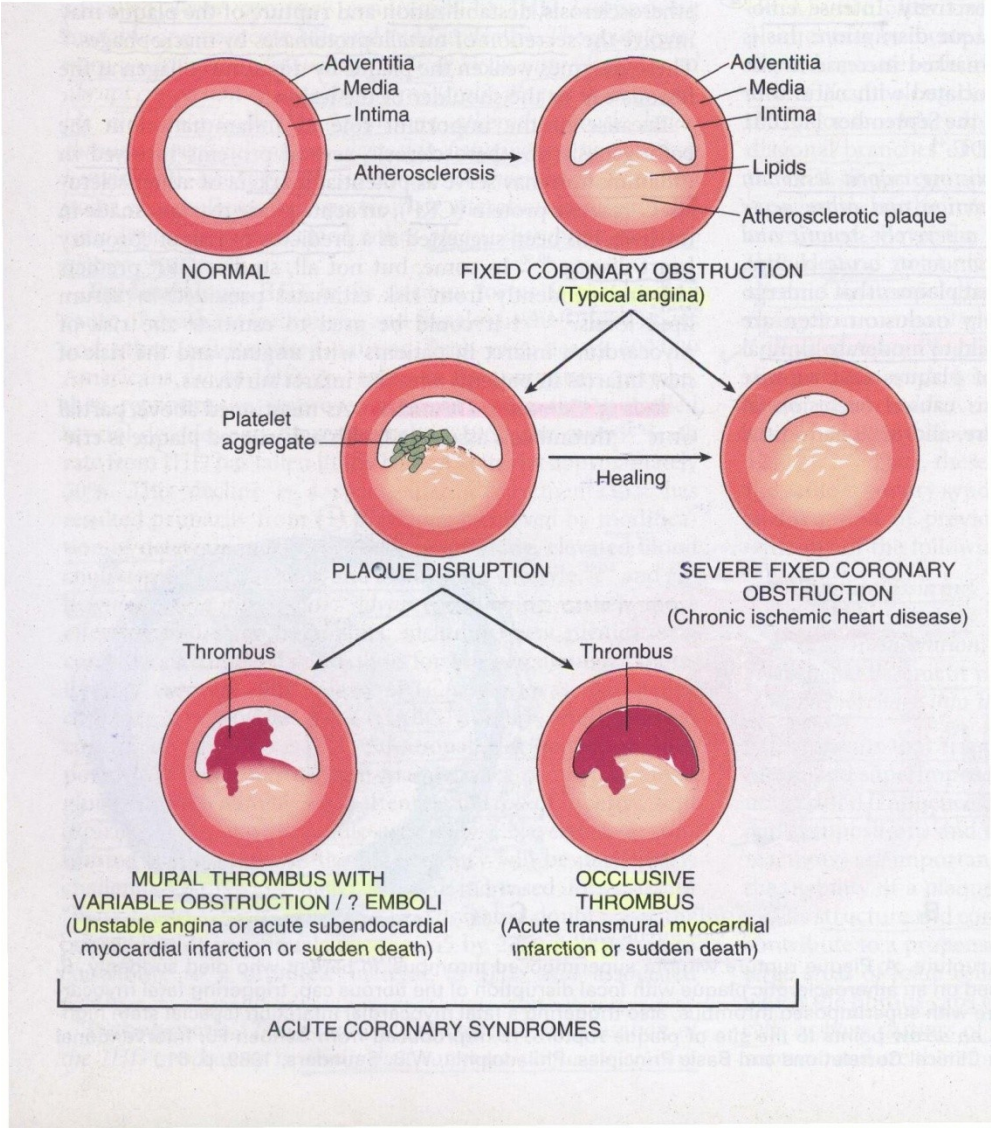
1) Rupture, fissuring or ulceration of plaques exposing highly thrombosed plaque constituents or underlying sub endothelial basement membrane.
2) Hemorrhage into the core of plaques with expansion of plaque volume and worsening of the limited occlusion [39, 22].

ROLE OF THROMBUS:-

Rupture of plaques fibrous cap causes thrombosis that leads to episodes of unstable angina [40].

Disruption of atherosclerotic plaque commonly causes arterial thrombosis by allowing blood coagulant factors to contact thrombogenic collagen found in the arterial Extra Cellular Matrix and tissue factor produced by macrophage derived foam cells in the lipid core of lesion. Thus the sites of plaque rupture form the nidus for thrombi.

CLINICAL TYPES OF ISCHEMIC HEART DISEASE



[From Kumar. V, Cotran. R, Robbins. S, Robbins basic pathology, edi-7, Philadelphia, 2003, Saunders, p574]

PATHO PHYSIOLOGY OF IHD :-

The extent of damage to myocardium and the irreversibility of the ischemic cardiac muscle depend on

1) The metabolic needs of the under perfused tissue.
2) Degree of existing collateral vessels.
3) Location, severity, duration and rate of development of arterial occlusion [41].

STABLE ANGINA :-

A lesion obstructing 70-75% or more of the vessel lumen causes symptomatic angina especially during increased demand.
A fixed stenosis of 90% can lead to diminished flow of blood even at rest [22].

ACUTE CORONARY SYNDROME (ACS) :-

The initiating event precipitating ACS is the alteration in structure of plaque leading to the development of a thrombus on top of an ulcerated or cracked atherosclerotic plaque. If the ensuring thrombus is transiently occlusive, the episode of plaque disruption may result in episodic ischemic symptoms with minimal myocardial damage (unstable angina) [26].

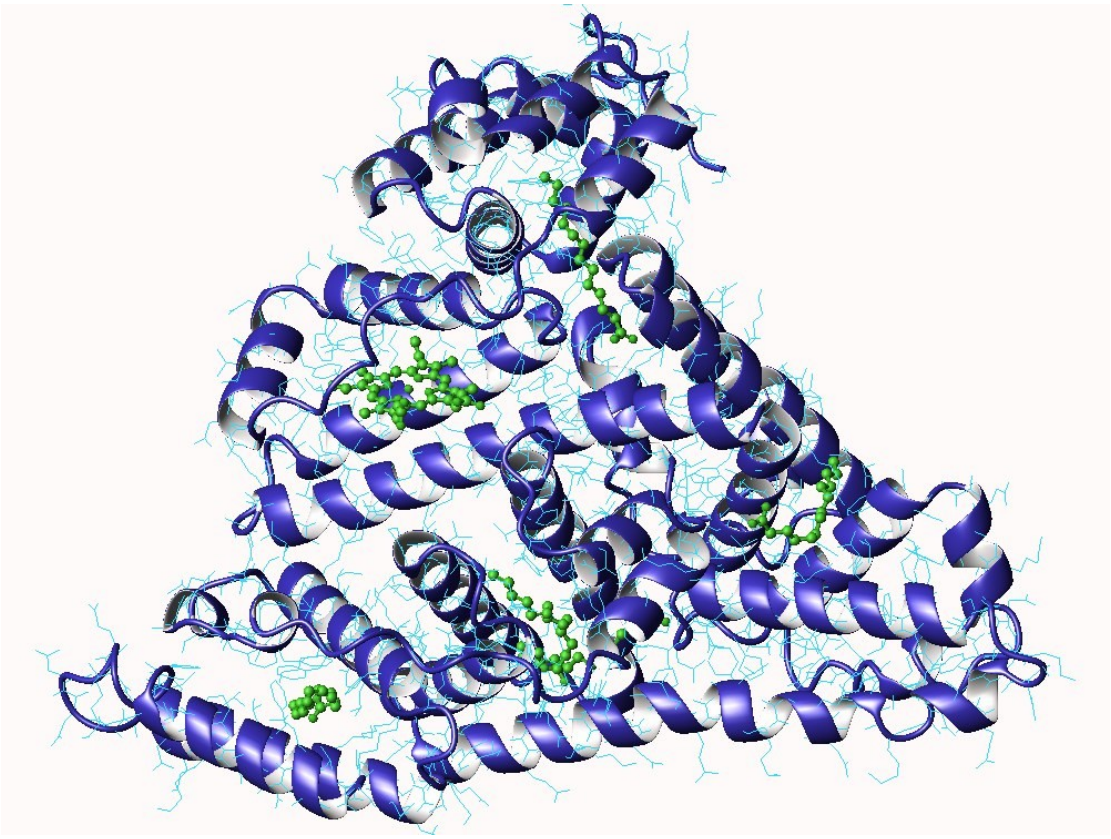
Acute myocardial infarction results from a severe atherosclerotic plaque occluding the coronary artery or from a relatively persistent thrombus over the ruptured plaque. It can be regional or diffuse. Regional Infarction is subdivided into transmural and sub endocardial [42].

Infarction spreads from the sub endocardial zone to the epicardium over a period of 4-6 hrs. Restoration of blood flow within this time will prevent a transmural infarct.

The introduction of thrombolytic therapy for patients with myocardial ischemia will reduce the infarct size, provided if the occlusion is due to thrombus, if the thrombus can be dissolved and if the occlusion has not been present for more than a few hours [9].

Animal experiments had also proved that reperfusion even up to 6 hours of coronary occlusion, resulted in a smaller infarct size, especially in the epicardium favouring early therapy to preserve ventricular function and to improve survival in myocardial ischemic patients[26].

STRUCTURE OF ALBUMIN



From Wikipedia – free encyclopedia

ALBUMIN:-

Albumin is the most abundant low molecular weight globular protein in plasma, 60% of which occupies the extravascular space[43].

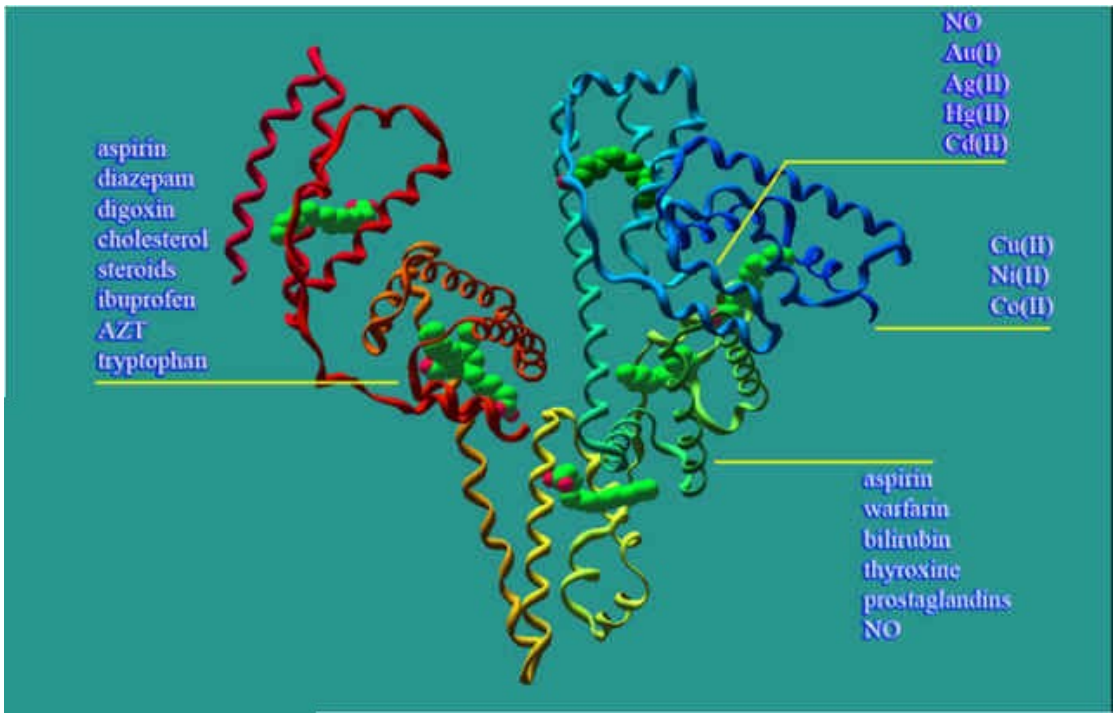
STRUCTURE:-

Albumin has a single polypeptide chain of 585 Amino acids, 35 cysteine residues, 34 of which forming 17 intra chain S – S bonds. The free SH group at position 34 of cysteine takes part in forming an inter molecular disulfide linkage[44] .N- Terminal end has an Amino acid sequence of DAHK (Aspartic acid, Alanine, Histidine, Lysine) [45,46]

BINDING SITES:-

- ✓ Site - I binds salicylates, Sulfanomides
- ✓ Site II – Tryptophan, thyroxine, octanoate
- ✓ Imidizole of His 3- forms a binding site for cu++ ions
- ✓ N terminal- binds metal ions like cobalt, nickel, copper [44].

BINDING SITES OF ALBUMIN



From Wikipedia – free encyclopedia

PROPERTIES:-

Albumin is a stable protein with high net negative charge. It's soluble in water. It has a plasma half life of 15-19 days[47].

METABOLISM:-

Albumin is synthesized by the hepatic parenchymal cells except in fetal life, where yolk sac is the primary site. Catabolism occurs by pinocytosis by all tissue and the resulting free amino acids are utilized for synthesis of cellular proteins. Small amounts are also lost into the gastro intestinal tract and the glomerular filtrate [43].

FUNCTION:-

1) Primary function is to maintain colloid oncotic pressure[48].
2) Able to bind and transport a large number of compounds like cholesterol, metallic ions, amino acids, drugs, hormones and bilirubin.
3) Acts as an amino acid source for peripheral tissue.
4) Albumin is an important component of plasma antioxidant activity.
5) It has buffering effect [49].

6) Increases capillary permeability to small proteins by binding to endothelial membrane associated glycoprotein.
7) Albumin reduces the inflammatory response of platelets and neutrophils by inhibiting leukotrienes [50].
8) It is essential for the metabolism and detoxification of many compounds.

CLINICAL SIGNIFICANCE:-

INCREASED PLASMA LEVELS:-

Occur as a consequence of dehydration due to reduced plasma water content leading to a general increase in plasma protein content.

Synthetic rate and intravascular-extra vascular shifts usually occur fairly rapidly to stabilize relative osmotic pressure [43].

DECREASED PLASMA LEVELS:-

1) Due to reduced synthesis as in liver disease [51].
2) Increased loss - in urine in nephrotic syndrome [52, 53]
   - Into intestine in protein losing enteropathy.
   - From the skin in burns.
   - In severe hemorrhage.

3. Impaired uptake in malnutrition.
4. Defective digestion or malabsorption [54,55].

ANALBUMINEMIA:-

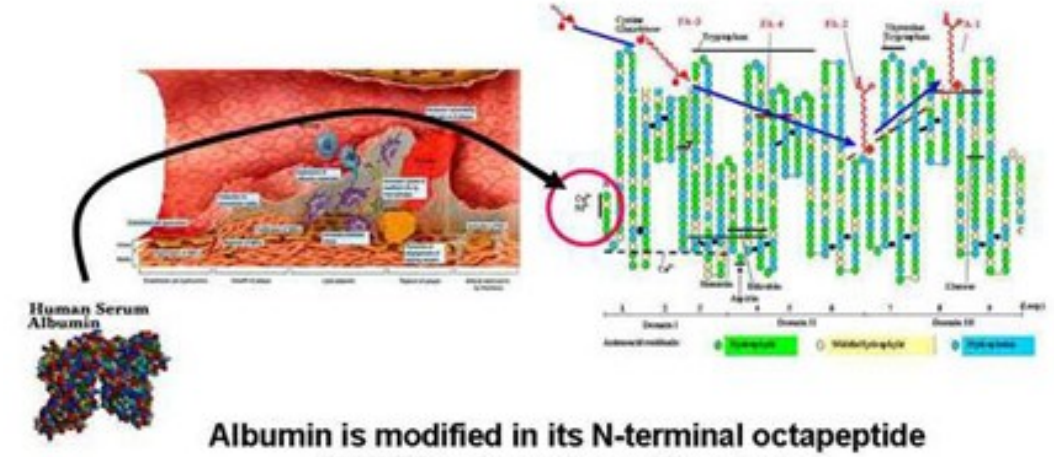
It's an inherited condition of plasma albumin with levels less than 0.5g/L[43].

REFERENCE LEVELS:-

35 to 52 g/l or

3.5 to 5.2 g/dl.

Ischemia Modified Albumin



Albumin is modified in its N-terminal octapeptide (DAHK) in hypoxic capillary beds, losing its affinity for Co 2+

From Wikipedia – free encyclopedia

ISCHEMIA MODIFIED ALBUMIN:-

In ischemia of the myocardium within seconds of vascular obstruction, aerobic glycolysis ceases in the myocytes, leading to inadequate production of adenosine triphosphate and depletion of creatine phosphate resulting in the accumulation of lactic acid, NADH and fall in pH [8.]

Cellular proteins and enzymes become progressively more dysfunctional as the pH falls. Depletion of ATP leads to reduction in the activity of the plasma membrane dependent sodium pump resulting in intracellular accumulation of sodium and efflux of potassium. Failure of the calcium pump leads to influx of calcium and its damaging effects [56,57].

Reduced pH leads to release of bound copper and iron from protein and intracellular stores. Ischemia also reduces the electron carriers, thereby leading to the formation of reactive oxygen species like super oxide anions [6,58,59].

These free radicals oxidatively damage the histidine present in the amino terminal region of albumin. This albumin which has a

damaged amino terminal is called Ischemia Modified Albumin (IMA) [60,61].

Normal albumin has a binding affinity for transitional metals like cobalt at its amino terminal. But Ischemia Modified Albumin lacks its ability to bind to cobalt which forms the basis for Albumin cobalt binding assay in measuring Ischemia Modified Albumin in myocardial ischemia [62].

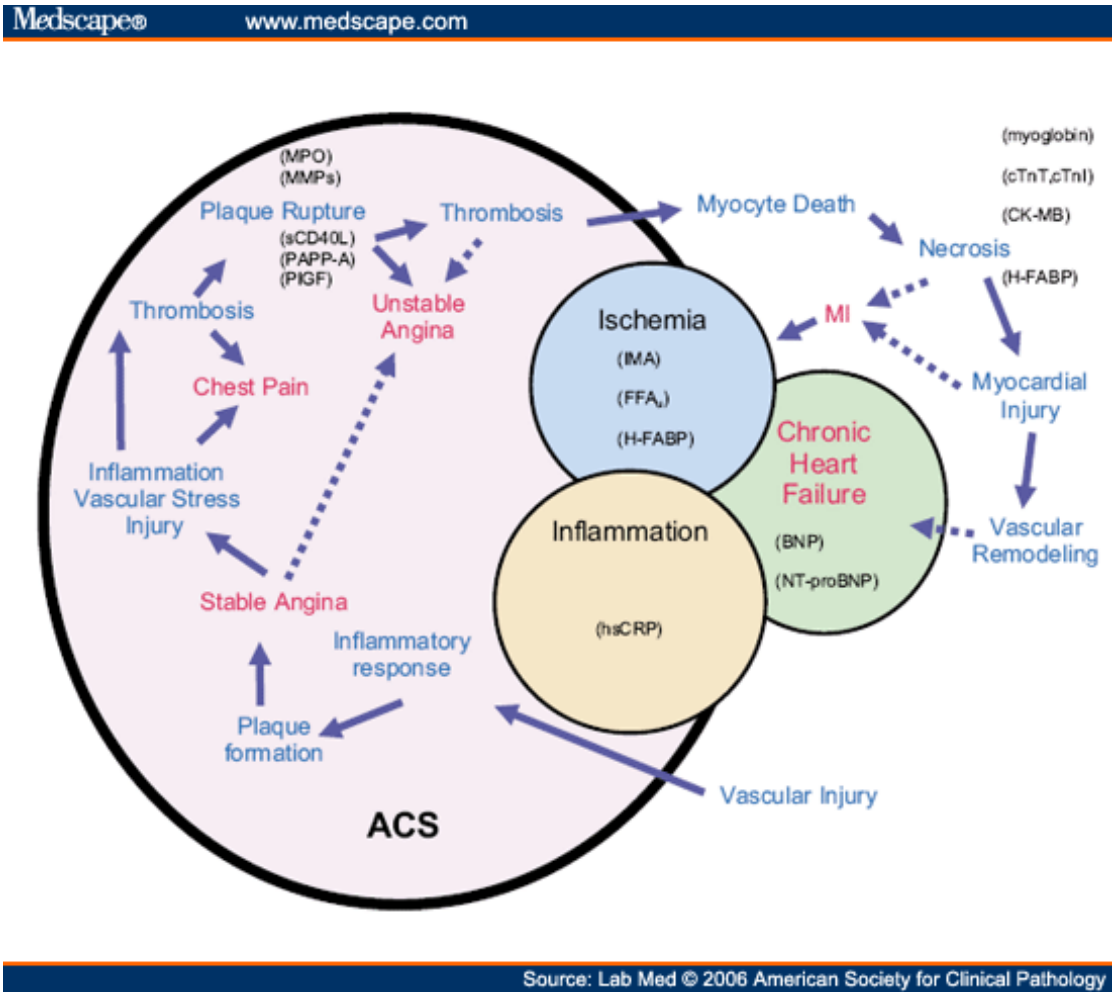
IMA starts increasing within 6 to 10 minutes of ischemia, reaches a peak by 4 hrs and returns to baseline after 6 hrs in transient ischemic conditions, like after Percutaneous transluminal angioplasty. Whereas the N-Terminal oxidative damage to albumin is cumulative and the repair is slow in cardiac ischemia, and the level also will not raise after 6 hours [59,63].

ISCHEMIA MODIFIED ALBUMIN:-

One Ischemia Modified Albumin unit (IMA Unit) is defined as "Microgram of free Cobalt in the reaction mixture per ml of Serum sample".

Normal values range from 6 – 80 u/ml.

RELATIONSHIP AMONG ACUTE CORONARY SYNDROME, INFLAMMATION, ISCHEMIA AND CHRONIC HEART FAILURE WITH ATHEROSCLEROSIS



From Update on Cardiac markers by Eileen Carreiro – Volume 37, No.10 October 2006, lab medicine

COBALT :-

Cobalt is an essential element for humans only as an integral part of vitamin B_{12} as a cofactor. No other function for Cobalt in human body is known.

Free Cobalt (non vitamin B_{12}) does not interact with body vitamin B_{12} pool. Intestinal micro flora cannot use Cobalt to synthesize physiologically active Cobalamine.

Deficiency: Cobalt deficiency has not been reported in humans.

Cobalt excess: Occupational over exposure to metal alloys containing Cobalt can lead to interstitial lung disease, cardiomyopathy and renal failure.

Cobalt is quantified in biological tissues by atomic absorption spectrometry[43].

MATERIALS AND METHODS:-

The study was conducted after getting the approval from the ethical committee of Stanley Medical College. Hundred subjects were chosen for the study. Both males and females in the age group of 30-70 years were included and an informed consent was obtained from all of them.

Fifty subjects with normal, clinical, biochemical parameters and with normal ECG served as the control group. They were selected from the master health check up outpatient department of Stanley Medical College.

Fifty subjects who were admitted in Intensive coronary care unit [ICCU] with complaints of chest pain (of < 6 hours duration), with Electro cardio graphic findings showing ST changes formed the study group and they were selected from the department of cardiology, Stanley Medical College.

INCLUSION CRITERIA:-

1) Patients admitted with complaint of chest pain within 6 hours of onset.

2) Electro cardio graphic findings showing abnormal ST-T wave changes (ST segment elevation or depression or deep symmetrical T wave inversion).

EXCLUSION CRITERIA:-

1) Presence of renal diseases.
2) Presence of cirrhosis,
3) Presence of stroke, skeletal muscle injury, malignancy, trauma.
4) Critically ill patients.
5) Ongoing infectious diseases.
6) Serum albumin < 2 gms/dl ,
   Serum creatinine > 3 mgs/dl.

BLOOD COLLECTION:-

5ml of blood samples were collected by vene puncture with strict aseptic precaution as soon as the subjects got admitted as per the inclusion criteria.

The samples were centrifuged and serum separated. One part of the sample was taken and analysis of CK-MB, albumin and

creatinine were done immediately. Remaining part of the sample was stored for analysis of Ischemia Modified Albumin at 20° C.

12-14 hours fasting sample was also collected from all subjects during their hospital stay and analysis of total cholesterol, triacylglycerol and high density lipoprotein were done.

ESTIMATION OF ISCHEMIA MODIFIED ALBUMIN

The tests were performed by chemical method using Cobalt chloride (hexa hydrate form) Dithiothreitol and Sodium chloride. All the chemicals were of Analytical reagent grade purchased from **SIGMA Chemicals**.

PRINCIPLE :-

Free Cobalt that does not bind to the Ischemia modified albumin in serum gives a brown coloured complex with Chromogen dithiothreitol which is measured spectrophotometrically at 470 nm.

Intensity of the colour is directly proportional to Ischemia modified albumin in serum.

REAGENTS :-

Cobalt chloride - 1 gm / litre

Dithiothreitol - 1.5 gm / litre

Sodium chloride - 9.0 gm / litre

All reagents are freshly prepared.

STANDARDIZATION OF THE PROCEDURE:-

Preparation of standards - Cobalt chloride [$CoCl_2.6H_2O$] was used for preparation of cobalt standards.

STOCK SOLUTION:- [1 gm % of cobalt]

Dissolve 0.183 mgs of cobalt chloride in deionised water and make up to 100ml.

SUBSTOCK:- [10,000 µg/10 ml of cobalt]

100 µl of stock solution is made up to 10ml.

WORKING STANDARDS:-

Working standards of various concentrations were prepared from the sub stock solution as shown in table.

Working standard concentration (µg/dl)	Stock solution (µl)	Deionised water (µl)
10	10	990
30	30	970
60	60	940
90	90	910
120	120	880

REAGENTS:-

1) **COBALT CHLORIDE :-**

0.183 mgs of Cobalt chloride dissolved in 100ml of deionised water.

2) **DITHIOSTHREITOL [DTT]:-**

1.5 gm of Dithiothreitol dissolved in .1 litre of deionised water.

3) 0.9 gms % SODIUM CHLORIDE:-

9.0 grams of Sodium chloride dissolved in 1 litre of deionised water to quench the reaction.

PROCEDURE:-

	Test	Standard	Blank
Sample	200µl	-	-
Standard (Cobalt chloride)	50µl	50µl	50µl
Deionised water	-	200µl	250µl
DTT	50µl	50µl	-
NaCl 0.9%	1ml	1ml	1ml

Add 50µl of cobalt chloride to the serum sample, mix vigorously and after ten minutes of incubation add 50 µl of Dithiothreitol. Wait for 2 minutes and then add 1ml of 0.9 % sodium chloride.

Read the absorbance at **470 nm** after 1 minute.

ABSORBANCE OF COBALT STANDARDS:-

Reagent blank = 0.030

STANDARD GRAPH - COBALT

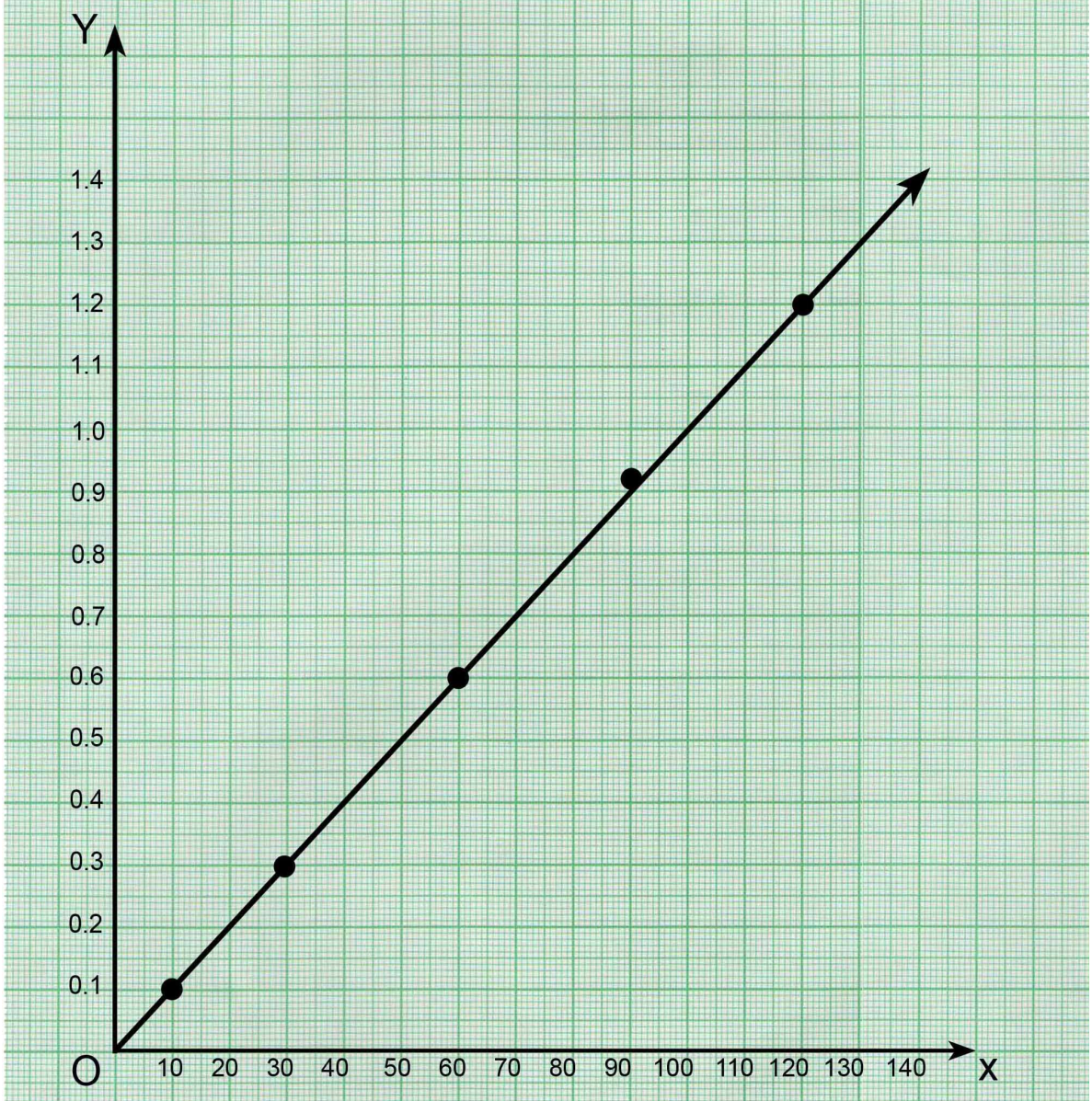
SCALE :-

X-AXIS - CONCENTRATION OF COBALT

1 cm - 10 micro gr

Y-AXIS - OPTICAL DENSITY

1 cm - 0.1 ABSORBANCE



Concentration of working standard (µg/dl)	Standard absorbance	Standard –Reagent blank absorbance
10	0.132	0.102
30	0.331	0.301
60	0.629	0.599
90	0.942	0.912
120	1.229	1.199

CALCULATION:-

Cobalt (µg / dl) = [**Ischemia modified albumin** units/ml]

$$\frac{\text{Absorbance of unknown}}{\text{Absorbance of standard}} \text{ x concentration of standard}$$

REFERENCE VALUES:-

6 – 80units/ml.

ESTIMATION OF SERUM (CK-MB)

The tests are performed in reagent kit by **Modified IFCC method.**

PRINCIPLE OF THE METHOD:-

This procedure involves measurement of CK activity in the presence of an antibody to CK-M monomer.

This antibody completely inhibits the activity of CK-MM and half of the activity of CK-MB while not affecting the B subunit activity of CK-MB and CK-BB. Then the CK method is used to quantitatively determine CK-B activity. The CK-MB activity is obtained by multiplying the CK-B activity by two.

Creatine phosphate + ADP $\xrightarrow{\text{creatine kinase}}$ Creatine + ATP

ATP + D-Glucose $\xrightarrow{\text{hexokinase}}$ Glucose-6-phosphate +ADP

Glucose-6-phosphate + NADP + $\xrightarrow{\text{Glucose 6 Phospate dehydrogenase}}$

6 - Phospogluconate + NADPH + H^+

REAGENTS:-

REAGENT I:- (Buffer / Enzymes)

REAGENT II:- (Polyclonal Antibody

WORKING REAGENT:-

Add 4ml of reagent I to one ml of reagent II. Mix gently by swirling till completely dissolved.

PROCEDURE:-

The reagent and sample are brought to room temperature.

To 1 ml of working reagent add 50 µl of sample and read immediately at **340** nm

	Blank	Standard	Test
Reagent	1 mL	1 mL	1 mL
Standard	--	50 µL	--
Sample	--	--	50µL

NORMAL VALUES:-

Serum: 0 – 24 u /L.

QUANTITATIVE DETERMINATION OF SERUM CREATININE

[MODIFIED JAFFE'S KINETIC METHOD]

PRINCIPLE OF THE METHOD:-

Creatinine present in the sample reacts with picric acid in alkaline medium forming creatinine picrate (red coloured complex) which is measured at 490 nm.

REAGENTS:-

Saturated picric acid	-	10 ml.
0.75 NaOH	-	10ml.
Distilled water	-	20 ml.

All reagents are mixed to prepare the working reagent.

PROCEDURE:-

Add 1 ml of working reagent to 100 µl of sample at 37^0C.

Mix and read at 490 nm

	Blank	Standard	Test
Reagent	1 mL	1 mL	1 mL
Standard	--	100 µL	--
Sample	--	--	100µL

Normal values: 0.8 – 1.2 mg /dl.

QUANTITATIVE DETERMINATION OF ALBUMIN:-
(BCG DYE BINDING METHOD):-

PRINCIPLE OF THE METHOD:-

Albumin in a buffered solution reacts with the anionic bromo cresol green (BCG) with a dye binding reaction to give a proportionate green colour which is measured at 630 nm (600 – 650 nm).

REAGENTS:-

Reagent I	- Bromocresol green
Succinic acid	- 94 m mol /L
Sodium hydroxide	- 10.2 m mol /L
BCG	:- 0.149 m mol /L.

PROCEDURE:-

The samples and the reagent were brought to room temperature before use.

Add 1 ml of reagent to 10 µl of sample, mix incubate for 1 minute at room temperature, and read at 630 nm.

	Blank	Standard	Test
Reagent	1 mL	1 mL	1 mL
Standard	--	10 µL	--
Sample	--	--	10µL

Normal values = 3.5 – 5 g / dl.

ESTIMATION OF TOTAL CHOLESTEROL:-

The tests are performed in the reagent kit by ENZYMATIC CHOLESTEROL ESTERASE METHOD.

PRINCIPLE;

The free cholesterol liberated from the cholesterol esters by cholesterol esterase is oxidized by cholesterol oxidase to cholestenone with the simultaneous production of hydrogen peroxide. The hydrogen peroxide reacts with 4 – amino antipyrine and a phenolic compound in the presence of peroxidase to yield a red coloured complex.

Cholesterol + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + Fatty acid

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol oxidase}}$ Cholestenone + H_2O_2

H_2O_2 + 4 - aminoantipyrine + Phenolic Compound

$\xrightarrow{\text{Peroxidase}}$ Quinone imine dye + H_2O

The concentration of cholesterol in the sample is directly proportional to the intensity of the red coloured complex which is measured at 500nm.

REAGENTS;

Reagent 1 (Enzymes / Chromogen)

Reagent 1A (Buffer)

STANDARD:-

Cholesterol 200 mg / dL

RECONSTITUTED REAGENT:-

Dissolve the contents of one bottle of reagent 1 with one bottle of reagent 1A.

PROCEDURE:-

	Blank	Standard	Test
Reagent	1 mL	1 mL	1 mL
Standard	--	10 µL	--
Sample	--	--	10µL

Incubate for 5 minutes at 37^0C.Read at 500 nm

NORMAL VALUES;

150-220mgs/dl

ESTIMATION OF TRIACYLGLYCEROL:-

The tests are performed in the reagent kit by ENZYMATIC COLORIMETRIC METHOD.

PRINCIPLE:-

Lipoprotein lipase catalyzed hydrolysis of triglycerides yield glycerol which is phosphorylated by Glycerol kinase using ATP to glycerol 3 phosphate which upon oxidation yields dihydroxy acetone phosphate and hydrogen peroxide. The hydrogen peroxide reacts with phenolic compound and 4-amino antipyrine to form a complex coloured.

Triacylglycerol + $H_2O \xrightarrow{\text{Lipase}}$ Glycerol + Free Fatty acids

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

Glycerol 3 Phosphate + O_2 $\xrightarrow{\text{Glycerol phosphate oxidase}}$ DAP + H_2O_2

H_2O_2+4 – Amino antipyrine +DHBS $\xrightarrow{\text{Peroxidase}}$ Quinoneimine dye + H_2O

DAP – Dihydroxy acetone phosphate

DHBS - 3,5 Dicholoro 2 – hydroxyl benzene sulfonate

The intensity of the purple coloured complex formed during the reaction is directly proportional to the triacylglycerol concentration in the sample and it is measured at 500 nm.

REAGENTS:-

REAGENT 1 (Enzymes / Chromogen)

REAGENT 1A (Buffer)

STANDARD:-

Triacylglycerol- 200 mg / dL.

RECONSTITUED REAGENT:-

Dissolve the contents of one bottle of reagent 1 with one bottle of reagent 1 A.

PROCEDURE:-

	Blank	Standard	Test
Reagent	1 mL	1 mL	1 mL
Standard	--	10 µL	--
Sample	--	--	10µL

Mix well and incubate for 5 minutes at 37^0C.and read at 500 nm.

NORMAL VALUES;

Male;-60-150 mg/dl

Female;-40-140 mg/dl

ESTIMATION OF HIGH DENSITY LIPOPROTEIN:-

The tests are performed in the reagent kit by PHOSPHOTUNGSTATE METHOD.

PRINCIPLE:-

Chylomicrons, very low density lipoprotein, low density lipoprotein fractions in serum or plasma are separated from high density lipoprotein (HDL) by precipitating with phosphotungstic acid and magnesium chloride. After centrifugation, the cholesterol in the HDL fraction, which remains in the supernatant, is assayed with enzymatic cholesterol method, using Cholesterol esterase, Peroxidase method.

REAGENTS:-

REAGENT 1 (Enzymes / Chromogen)

REAGENT 1A (Buffer)

REAGENT 2 (Precipitating Reagent)

Phosphotungstic acid	50 m mol / L
Magnesium Chloride	39 m mol /L

STANDARD:-

HDL Cholesterol- 50 mg / dL.

PROCEDURE:-

1. Precipitation

Dispense into centrifuge tubes.

	Test
Sample	200 µL
Precipitating reagent	200 µL

Mix well. Centrifuge at 3500 – 4500 rpm for 10 minutes. Separate the clear supernatant immediately and determine the cholesterol content.

PROCEDURE:-

	Blank	Standard	Test
Reagent	1 mL	1 mL	1 mL
Standard	--	10 µL	--
Sample	--	--	10µL

Mix well and incubate for 5 minutes at 37^0C.and read at 500 nm

NORMAL VALUES: 40-70 mgs/dl

CALCULATED PARAMETERS:-

FRIEDWALD'S FORMULA:

VERY LOW DENSITY LIPOPROTEIN: $\frac{\text{Triacylglycerol}}{5}$

LOW DENSITY LIPOPROTEIN:

Total Cholesterol – HDL – $\frac{\text{Triacylglycerol}}{5}$

RESULTS AND STATISTICAL ANALYSIS

A total of 100 patients were included in the present study. Out of the 100, 50 were study group [IHD patients within 6 hours of onset of Chest pain] and other 50 were controls [Normal individuals].

AGE DISTRIBUTION AMONG THE STUDY AND CONTROL GROUP :-

Male and Female patients in the age group of 35 years to 70 years were taken in the study. Both the study and control group were age matched.

The mean age of the control group is 51.48 and the mean age of the study group is 52.04.

TABLE – I

Group	N	Minimum age	Maximum age	Mean	Standard Deviation	Student independent 't' test
Control	50	35	67	51.48	8.853	P=0.09
Study	50	35	68	52.04	9.178	Not Significant

Quantitative variable [age] is given as frequency with their percentages.

TABLE – II

Age [years]	Control		Study		Chi Square test
	Number	Percentage	Number	Percentage	
Upto 40	8	16.0	8	16.0	P=0.31 Not significant
40 – 50	15	30.0	15	30.0	
51 – 60	17	34.0	16	32.0	
>61	10	20.0	11	22.0	
	50	100.0	50	100.0	

The serum levels of total cholesterol, Triacylglycerol and High density lipoprotein were estimated for all the patients taken for the study. Very low density lipoprotein and low density lipoprotein values were calculated.

The values obtained in both the study and control group are presented in the master chart I & II.

Mean and standard deviation were calculated for the quantitative variables, Total cholesterol, Triacylglycerol, High density

lipoprotein, very low density lipoprotein and low density lipoprotein, IMA & CK-MB, in both study and control group and also in male and female patients separately. The values were analysed and the results are presented in tables 3 – 9.

Correlation between IMA and Ck-MB were analyzed using pearson's correlation analysis. The results are presented in table 10 & 11.

The Receiver operating characteristic [ROC] Curves are plotted for IMA and CK-MB and presented in figures I & II.

COMPARISON OF BIOCHEMICAL PARAMETERS IN THE STUDY AND CONTROL GROUP

TABLE – III

Parameter	Group	Mean	Standard deviation	'P' value
IMA	Control Study	38.35 88.52	13.95 26.57	P=0.001 Significant
CK-MB	Control Study	12.32 56.50	4.23 49.21	P=0.032 Significant
Total cholesterol	Control Study	166.12 208.44	17.67 37.29	P=0.04 Significant
Triacyl glycerol	Control Study	125.45 128.38	24.25 16.94	P=0.058 Significant
High density lipoprotein	Control Study	44.04 34.44	9.53 8.43	P=0.01 Significant
Low density lipoprotein	Control Study	96.00 148.26	22.64 35.18	P=0.032 Significant
Very low density lipoprotein	Control Study	24.96 25.70	4.91 3.40	P=0.05 Significant

COMPARISON OF BIOCHEMICAL PARAMETERS IN THE AGE MATCHED STUDY AND CONTROL GROUP

TABLE – IV

[AGE < 40 YEARS]

Parameter	Group	Mean	Standard deviation	'P' value
IMA	Control Study	40.68 98.31	17.91 21.60	P=0.001 Significant
CK-MB	Control Study	11.00 63.37	4.65 51.20	P=0.05 Significant
Total cholesterol	Control Study	177.37 229.62	10.47 39.85	P=0.02 Significant
Triacyl glycerol	Control Study	122.87 134.50	23.78 24.69	P=0.07 Not Significant
High density lipoprotein	Control Study	42.37 33.62	12.87 8.97	P=0.03 Significant
Low density lipoprotein	Control Study	109.50 169.00	21.17 41.47	P=0.04 Significant
Very low density lipoprotein	Control Study	24.25 27.00	5.06 4.78	P=0.11 Not Significant

COMPARISON OF BIOCHEMICAL PARAMETERS IN THE AGE MATCHED STUDY AND CONTROL GROUP

TABLE – V

[AGE 41 – 50 YEARS]

Parameter	Group	Mean	Standard deviation	'P' value
IMA	Control Study	34.80 95.33	13.42 20.27	P=0.01 Significant
CK-MB	Control Study	12.40 52.33	4.51 55.60	P=0.04 Significant
Total cholesterol	Control Study	166.46 202.80	14.54 40.69	P=0.021 Significant
Triacyl glycerol	Control Study	121.13 129.13	30.74 19.02	P=0.05 Significant
High density lipoprotein	Control Study	41.86 32.20	9.07 9.61	P=0.16 Not Significant
Low density lipoprotein	Control Study	97.46 144.73	22.55 38.16	P=0.04 Significant
Very low density lipoprotein	Control Study	24.33 25.73	6.07 3.95	P=0.64 Not Significant

COMPARISON OF BIOCHEMICAL PARAMETERS IN THE AGE MATCHED STUDY AND CONTROL GROUP

TABLE – VI

[AGE 51 – 60 YEARS]

Parameter	Group	Mean	Standard deviation	'P' value
IMA	Control Study	36.05 67.75	13.15 30.55	P=0.001 Significant
CK-MB	Control Study	12.35 50.12	4.09 42.54	P=0.05 Significant
Total cholesterol	Control Study	160.41 214.12	20.15 30.58	P=0.04 Significant
Triacyl glycerol	Control Study	127.00 126.06	22.70 13.28	P=0.19 Not Significant
High density lipoprotein	Control Study	45.29 36.00	8.63 7.78	P=0.05 Significant
Low density lipoprotein	Control Study	88.70 152.75	23.41 28.66	P=0.03 Significant
Very low density lipoprotein	Control Study	25.11 25.31	4.72 2.67	P=0.03 Significant

COMPARISON OF BIOCHEMICAL PARAMETERS IN THE AGE MATCHED STUDY AND CONTROL GROUP

TABLE – VII

[AGE > 60 YEARS]

Parameter	Group	Mean	Standard deviation	'P' value
IMA	Control Study	45.72 102.30	11.20 10.58	P=0.01 Significant
CK-MB	Control Study	13.20 66.45	4.07 52.43	P=0.05 Significant
Total cholesterol	Control Study	166.30 192.45	19.75 35.27	P=0.017 Significant
Triacyl glycerol	Control Study	131.20 126.27	17.26 12.82	P=0.76 Not Significant
High density lipoprotein	Control Study	46.50 35.81	9.21 7.58	P=0.04 Significant
Low density lipoprotein	Control Study	95.40 131.45	20.27 29.66	P=0.62 Not Significant
Very low density lipoprotein	Control Study	26.20 25.27	3.39 2.53	P=0.03 Significant

COMPARISON OF BIOCHEMICAL PARAMETERS IN THE SEX MATCHED STUDY AND CONTROL GROUP

TABLE – VIII

MALE

Parameter	Group	Mean	Standard deviation	'P' value
IMA	Control Study	35.50 92.91	12.97 23.88	P=0.01 Significant
CK-MB	Control Study	11.53 51.55	3.99 45.61	P=0.03 Significant
Total cholesterol	Control Study	164.70 205.15	17.43 32.97	P=0.04 Significant
Triacyl glycerol	Control Study	120.83 123.00	26.65 16.86	P=0.16 Not Significant
High density lipoprotein	Control Study	44.86 37.20	10.85 8.01	P=0.05 Significant
Low density lipoprotein	Control Study	93.36 151.60	25.31 37.84	P=0.05 Significant
Very low density lipoprotein	Control Study	24.00 26.43	5.31 3.26	P=0.07 Not Significant

COMPARISON OF BIOCHEMICAL PARAMETERS IN THE SEX MATCHED STUDY AND CONTROL GROUP

TABLE – IX

FEMALE

Parameter	Group	Mean	Standard deviation	'P' value
IMA	Control Study	42.62 92.91	14.59 23.88	P=0.04 Significant
CK-MB	Control Study	13.50 51.55	4.39 45.61	P=0.05 Significant
Total cholesterol	Control Study	168.25 205.15	18.26 32.97	P=0.04 Significant
Triacyl glycerol	Control Study	132.30 123.00	18.71 16.86	P=0.03 Significant
High density lipoprotein	Control Study	42.80 37.20	7.19 8.01	P=0.03 Significant
Low density lipoprotein	Control Study	99.95 143.25	17.80 31.00	P=0.17 Not Significant
Very low density lipoprotein	Control Study	26.40 24.60	3.95 3.39	P=0.14 Not Significant

PEARSON'S CORRELATION ANALYSIS

IMA WITH OTHER VARIABLES

TABLE – X

IMA	Age	CK-MB	TC	TAG	HDL	LDL	VLDL
Correlation 1	0.162	0.53	0.482	0.062	0.374	0.55	0.459
Significance [2 tailed]	0.031	<0.001	0.004	0.06	0.001	0.002	0.56
	S	S	S	NS	S	S	NS

CK-MB WITH OTHER VARIABLES

TABLE – XI

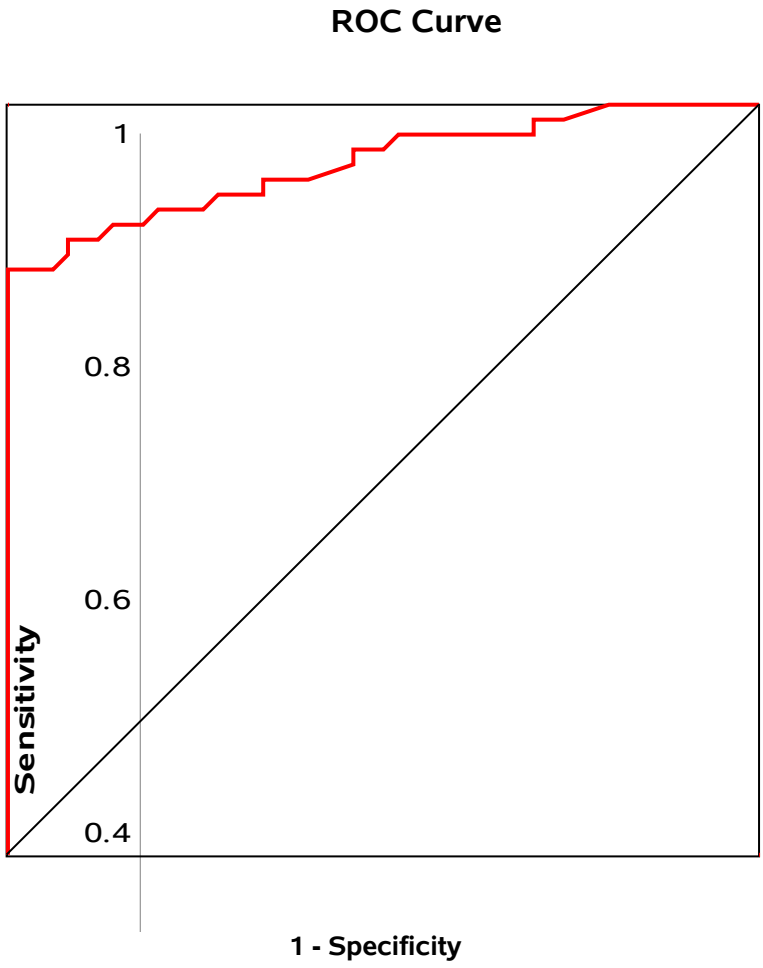
CK-MB	Age	CK-MB	TC	TAG	HDL	LDL	VLDL
Correlation 1	0.127	0.53	0.321	0.008	0.17	0.342	0.009
Significance [2 tailed]	0.042	<0.001	0.001	0.038	0.091	0.001	0.928
	S	S	S	S	NS	S	NS

S - Significant

NS - Not Significant

RECEIVER OPERTING CHARACTERISTIC CURVE FOR

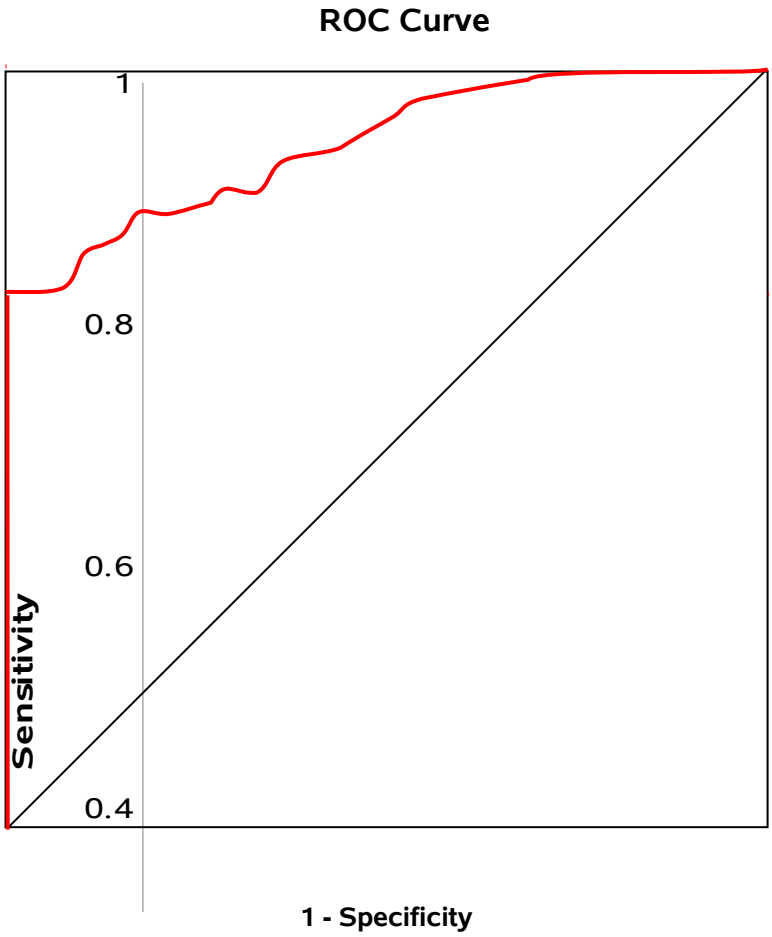
ISCHEMIA MODIFIED ALBUMIN (IMA)



DIAGONAL SEGMENTS ARE PRODUCED BY TIES

RECEIVER OPERTING CHARACTERISTIC CURVE FOR

CK-MB



DIAGONAL SEGMENTS ARE PRODUCED BY TIES

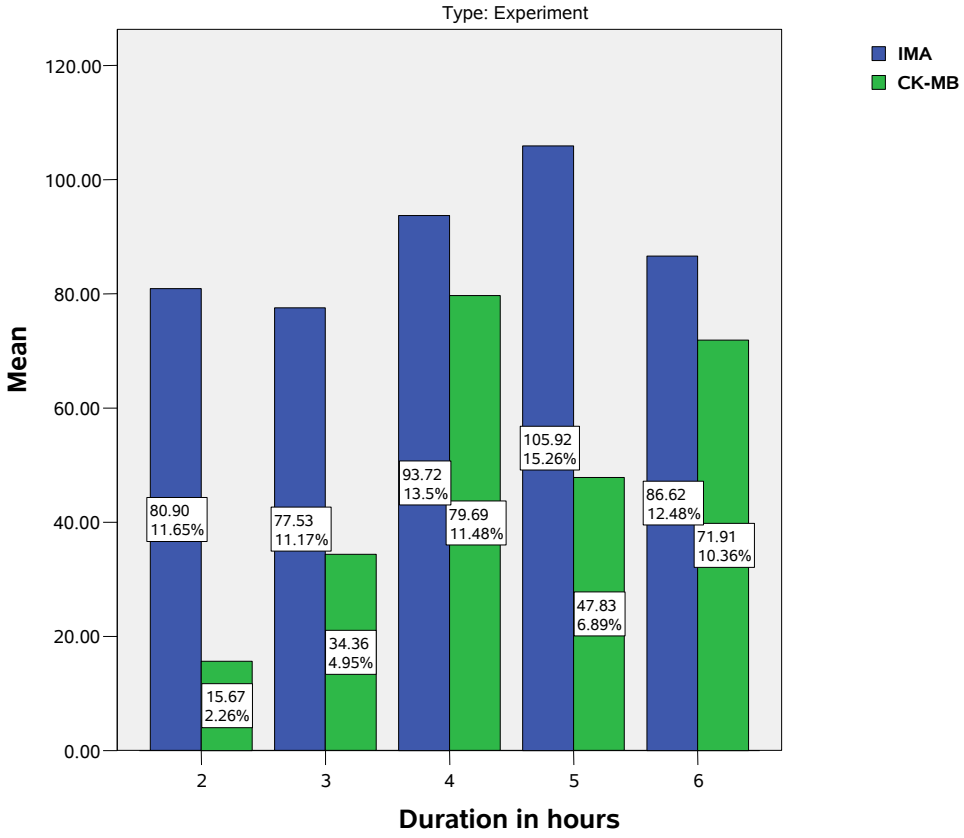
DISCUSSION

The present study establishes the characterization of the IMA test for its association in the early diagnosis of Myocardial Ischemic patients and its comparison with CK-MB, the common biochemical marker of Coronary Heart Disease.

The ability to detect ischemia before myocyte destruction is necessary for earlier and more accurate management decisions for the patients suspected to have Acute Coronary Syndrome. The current possible tests [58] like CK-MB, serum Troponin-I and Myoglobin levels will be detected in serum, only after considerable myocardial cell death and necrosis had occurred, which are precipitating factors for morbidity and mortality [58,64].

In the present study, the mean value of IMA of 88.52units/ml in the study group showed a significant rise than CK-MB during the early hours of ischemia with a sensitivity of 78%. This study also correlates with the data given in previous studies on Ischemia Modified Albumin-as a novel marker of Acute Coronary Syndrome[57].

IMA / CK-MB LEVELS WITH DURATION OF CHEST PAIN



BAR DIAGRAM FIGURE - 1

Traditional risk factors used in the prediction of atherosclerosis are Total Cholesterol, Triacylglycerol and low density lipoprotein while High density lipoprotein is a marker of anti atherogenic potential in an individual.

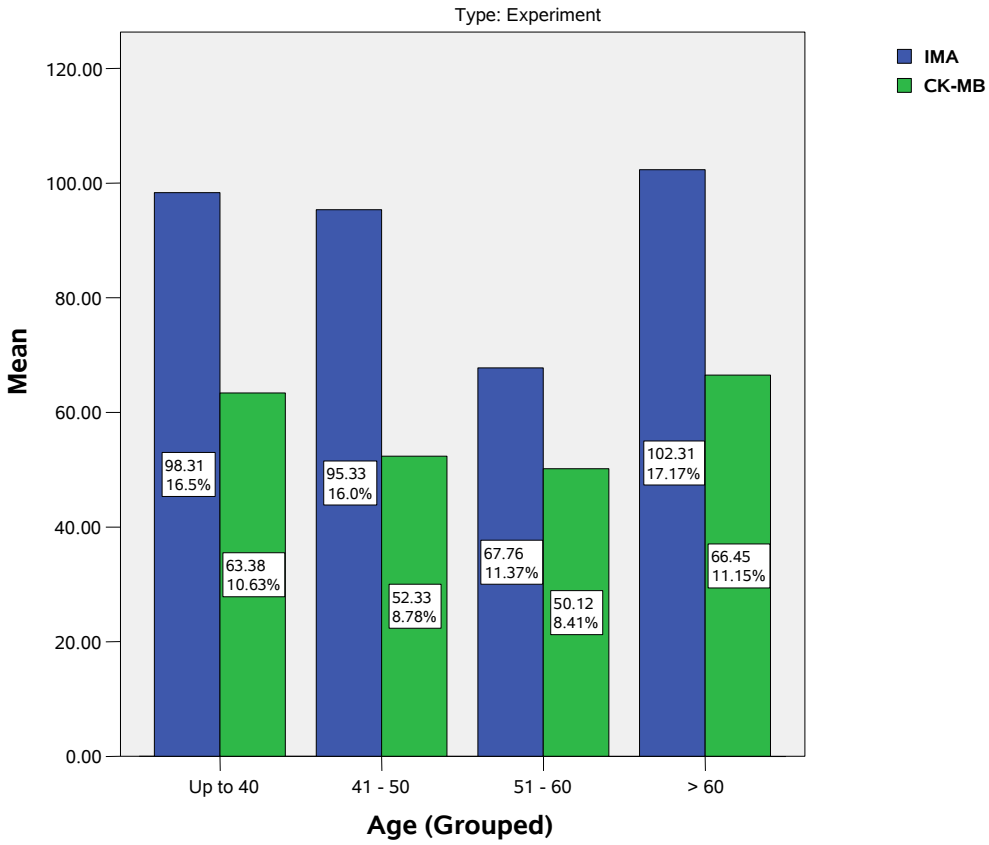
Possible association of other modifiable risk factors like Hypertension, Diabetes, Smoking and alcohol consumption also necessitates the predilection for a serious outcome.

Various epidemiological and clinical studies have shown strong relationship between IMA and CK-MB levels increase in relationship to duration of chest pain [57].

The present study also shows a strong positive correlation with duration of chest pain which is depicted in the bar diagram in (Fig-1). There is significant serial elevation in IMA levels from 2 hours to 6 hours of onset of chest pain compared to the percent increase in CK-MB levels.

ROC curve of IMA and CK-MB reveal that the IMA curve is above the assay curve of CK-MB [1, 65].

IMA / CK-MB LEVELS WITH AGE GROUP



BAR DIAGRAM FIGURE - 2

AREA UNDER THE CURVE;

Test result variable (IMA):

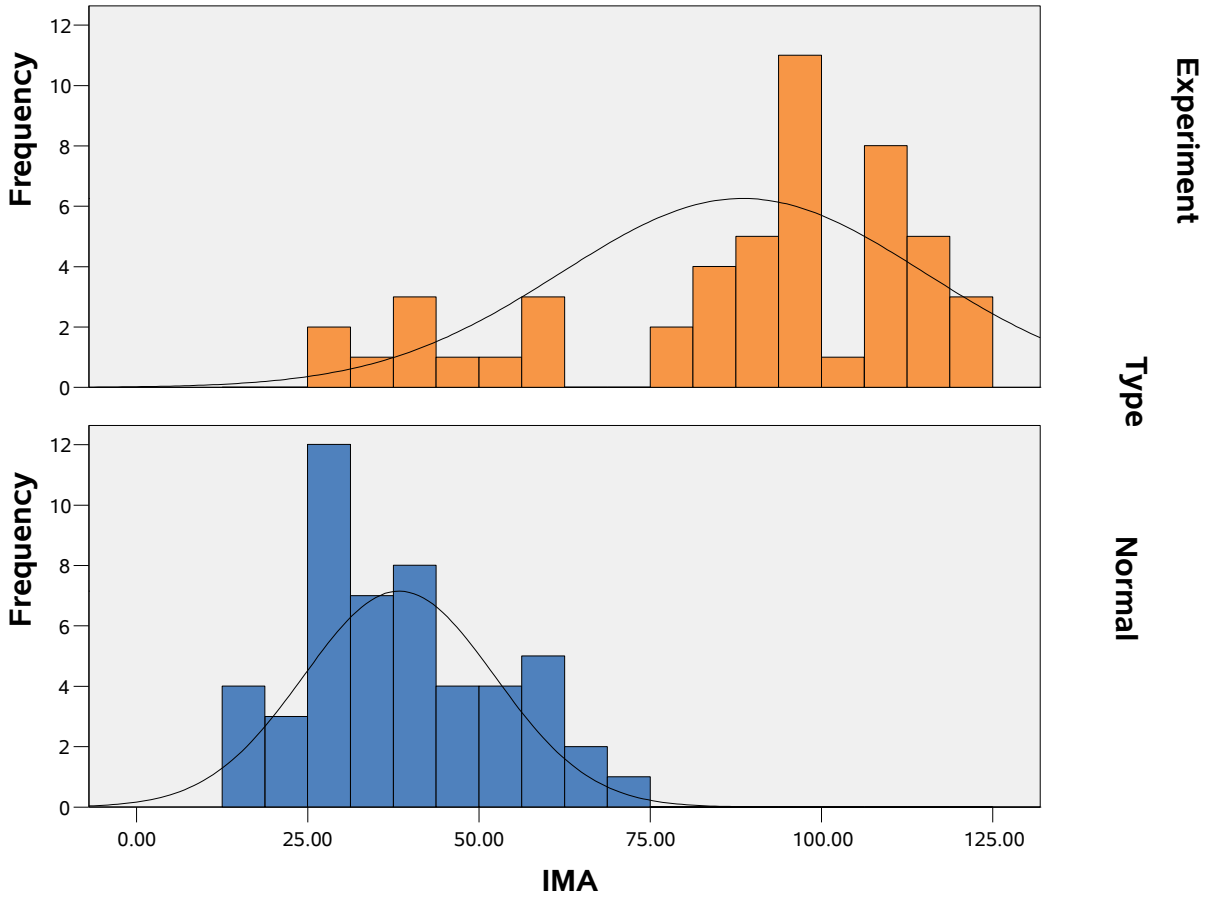
Area	Std error	Asym sig	Asymptomatic 95% confidence interval	
			Low bound	Upper bound
0.921	0.28	0.000	0.866	0.976

With an area under the curve of 0.921 and a standard error of 0.028, IMA showed an asymptomatic significance of 0.000 proving it as a better predictor over CK-MB, in diagnosing myocardial ischemia. Hence IMA is a better assay for evaluating ischemia before CK-MB.

The Bar chart in fig 2 shows the increase in IMA levels with relation to the age group.

When the mean values of IMA and CK-MB are compared in the study and control groups, a significant relationship exists between both the study and control groups in all age group especially in the age group of < 40 years, 41-50 years and > 60 years.

IMA LEVELS IN NORMAL (CONTROL) AND EXPERIMENT (STUDY) GROUPS



RISK FACTOR ASSOCIATION:

Age group(years)	Total number	Smoking	Alcohol	HT	DM
<40	8	4	4	2	1
41-50	15	7	5	11	1
51-60	16	2	1	11	5
>60	11	4	1	9	7

Comparing the presence of risk factors, myocardial ischemia in <40 years can be attributed to smoking and alcohol consumption in this age group.

Onset of HT and Diabetes in addition, contributed to the precipitation of myocardial ischemia in patients over the age group of 40 years.

The difference in the age matched study group did not show a uniform significance in any of the measured parameters except IMA and CK-MB. Mean value of total cholesterol shows a significant elevation in all age groups and in both male and female. Triacylglycerol is raised in the age group of 41 to 50 years.

The decrease in High Density Lipoprotein in the age group of 41 to 50 years shows non significance where as it is significant in other age groups. Level of low density lipoprotein significantly elevated in<40 years, 41 to 50 years and 51 to 60 years where as VLDL is markedly significant in 51 to 60 years and more than 60 years age group.

Though there is not much elevation in the mean of the measured parameters in both male and female, only a slight increase occurs in male patients compared to female patients. This can be associated to the protective effects of hormones in female patients before menopause.

In the Pearson's correlation analysis, there is a significant correlation noticed between IMA and CK-MB with age, Total cholesterol and LDL. Progressive atherosclerosis with increasing age leads to ischemia with super added risk factors precipitating the development of atherosclerosis at an early age.

Alteration in food habits and life style changes can decrease the effects of modifiable risk factors over atherosclerosis which can delay the onset of ischemia.

LIMITATIONS OF THE STUDY:

1) Albumin Cobalt binding (ACB) assay which is utilized to estimate IMA levels in ischemic patients is based on the modification in the amino terminal region of albumin produced by extra cellular hypoxia, acidosis, and a free radical injury disruption[66]. Therefore ischemia in the absence of necrosis may cause bias towards apparent false positive Albumin Cobalt binding data.

2) The currently used colorimetric Albumin Cobalt binding assay is an indirect measurement of IMA production. New assay platforms (Immuno assays) are expected to be available in future which may improve the specificity of IMA [66, 67].

3) Currently no reference standard exists for cardiac ischemia. A combination test of IMA with CK-MB and Troponin I can increase the sensitivity in the early diagnosis of Acute Coronary Syndrome [56, 68].

4) Time at which blood sample is collected in relation to the onset of chest pain and other conditions that cause false positive and negative results must be established[69].

CONCLUSION:

Biochemical markers such as CK-MB, Cardiac Troponin-I and Myoglobin are suitable only for assessing myocardial infarction. The results of the present study confirm the findings of previous studies, that reported that the Albumin Cobalt colorimetric assay distinguishes myocardial ischemic patients from non ischemic patients ($p<0.001$)

Introduction of IMA assay for the first time provides emergency physicians with an objective diagnostic study to determine the presence of myocardial ischemia completely within the control of the emergency department.

IMA assay presents a quantitative accurate laboratory determination of the occurrence of an Ischemic myocardial event, Angina of various types.

Unlike the previous laboratory parameters that identify myocardial damage, only after it is well established, this test (Albumin Cobalt

binding assay) helps to determine which patients will go in for severe occlusion.

The introduction of IMA is a welcome event and based on the results obtained, the present study supports the hypothesis that Ischemia Modified Albumin is a useful marker for the early diagnosis of myocardial ischemia before any significant increase in CK-MB levels.

SCOPE FOR FUTURE STUDY

IMA level estimation in outpatient department itself can be done in patients complaining of chest pain to rule out myocardial ischemia before ECG manifestation especially in unstable angina, and other variants of Angina like Nocturnal Angina, prinzmetal's variant Angina and stable exertional Angina.

It can be also used to rule out non anginal causes of chest pain.

If these observations are confirmed, IMA can be used as an outpatient investigation tool, to reduce in appropriate Hospital Admissions of Low risk patients.

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MASTER CHART FOR STUDY GROUP

Sl. No.	AGE	SEX	Duration	IMA u/ml	CkMB U/ltr	Creatinine mg/dl	Albumin gm/dl	TC mg/dl	TGL mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
1	58	F	3	84.50	18.00	0.70	3.80	238.00	139.00	30.00	180.00	28.00
2	40	F	6	106.50	96.00	0.80	4.20	258.00	114.00	39.00	196.00	23.00
3	62	F	4	110.20	138.00	0.80	3.60	216.00	124.00	47.00	144.00	25.00
4	68	F	4	98.60	56.00	0.60	3.80	192.00	106.00	34.00	137.00	21.00
5	56	F	3	94.30	114.00	0.70	3.50	213.00	132.00	46.00	141.00	26.00
6	65	F	4	88.30	30.00	0.50	4.00	144.00	137.00	26.00	91.00	27.00
7	55	F	3	38.40	46.00	0.60	3.80	200.00	117.00	36.00	141.00	23.00
8	61	F	6	114.60	12.00	0.70	3.60	227.00	114.00	41.00	163.00	24.00
9	56	F	5	118.00	4.00	0.80	4.00	189.00	119.00	22.00	143.00	24.00
10	45	F	2	122.60	8.00	0.70	3.80	222.00	126.00	33.00	164.00	25.00
11	38	F	3	56.60	22.00	0.80	4.00	151.00	103.00	36.00	94.00	21.00
12	50	F	3	92.20	10.00	0.70	3.60	192.00	115.00	36.00	133.00	21.00
13	66	F	4	106.40	12.00	0.60	3.80	185.00	122.00	36.00	125.00	24.00
14	48	F	5	112.30	16.00	0.80	4.00	189.00	180.00	22.00	131.00	36.00
15	60	F	4	95.40	154.00	0.70	3.60	259.00	114.00	39.00	196.00	23.00
16	52	F	6	82.30	66.00	0.80	3.80	202.00	104.00	52.00	129.00	21.00
17	40	F	4	79.60	52.00	0.80	3.50	216.00	124.00	47.00	144.00	25.00
18	55	F	6	98.30	78.00	0.70	3.80	178.00	124.00	40.00	113.00	25.00
19	44	F	5	116.40	81.00	0.60	3.60	172.00	113.00	41.00	108.00	23.00
20	52	F	2	42.70	18.00	0.70	3.80	260.00	133.00	41.00	192.00	27.00
21	44	M	4	122.50	90.00	0.70	3.80	166.00	134.00	22.00	117.00	27.00
22	39	M	3	98.60	15.00	0.80	4.20	276.00	176.00	20.00	221.00	35.00
23	54	M	6	28.40	28.00	0.60	3.10	189.00	119.00	33.00	132.00	24.00
24	67	M	4	112.30	136.00	0.70	3.80	194.00	134.00	37.00	130.00	27.00
25	49	M	2	52.80	18.00	0.60	4.00	227.00	136.00	22.00	178.00	27.00
26	40	M	3	117.40	45.00	0.80	3.50	236.00	136.00	33.00	176.00	27.00
27	35	M	4	114.20	52.00	0.60	3.60	241.00	125.00	22.00	194.00	25.00
28	67	M	2	108.60	24.00	0.70	3.80	203.00	120.00	40.00	139.00	24.00
29	40	M	4	120.00	175.00	0.80	3.90	201.00	165.00	33.00	135.00	33.00
30	63	M	6	102.00	80.00	0.70	4.00	264.00	143.00	43.00	192.00	29.00
31	60	M	2	60.30	12.00	0.80	3.60	241.00	133.00	31.00	183.00	27.00
32	62	M	4	98.20	36.00	0.80	3.60	144.00	137.00	26.00	91.00	27.00
33	46	M	4	89.60	28.00	0.70	3.80	299.00	144.00	51.00	219.00	29.00
34	47	M	3	85.10	10.00	0.80	3.60	213.00	132.00	46.00	141.00	26.00
35	45	M	6	89.40	142.00	0.70	3.50	251.00	123.00	27.00	199.00	25.00
36	58	M	3	28.70	56.00	0.60	3.70	179.00	160.00	31.00	116.00	32.00
37	52	M	5	96.20	8.00	0.70	3.60	226.00	127.00	38.00	163.00	25.00
38	42	M	6	97.50	165.00	0.80	3.60	189.00	119.00	22.00	143.00	24.00
39	56	M	4	35.40	38.00	0.60	3.80	177.00	134.00	30.00	120.00	27.00
40	35	M	6	93.60	50.00	0.50	3.60	258.00	133.00	39.00	192.00	27.00
41	48	M	2	98.40	14.00	0.80	3.50	135.00	145.00	33.00	73.00	29.00
42	45	M	3	110.60	20.00	0.60	3.90	205.00	101.00	37.00	148.00	20.00
43	52	M	4	38.60	98.00	0.70	3.80	260.00	133.00	41.00	192.00	27.00
44	65	M	6	79.80	56.00	0.70	3.50	178.00	141.00	24.00	126.00	28.00
45	48	M	5	86.20	27.00	0.50	4.00	238.00	139.00	30.00	180.00	28.00
46	50	M	4	94.00	138.00	0.70	3.90	170.00	111.00	40.00	108.00	22.00
47	52	M	3	46.40	22.00	0.60	3.70	227.00	114.00	41.00	163.00	23.00
48	58	M	4	96.20	42.00	0.50	3.80	188.00	115.00	25.00	140.00	23.00
49	64	M	5	106.40	151.00	0.60	3.80	170.00	111.00	40.00	108.00	22.00
50	48	M	6	60.40	18.00	0.70	3.60	174.00	119.00	21.00	129.00	24.00

MASTER CHART FOR CONTROL GROUP

Sl. No.	AGE	SEX	IMA u/ml	CkMB U/ltr	Creatinine mg/dl	Albumin gm/dl	TC mg/dl	TGL mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl

1	52	F	38.60	12.00	0.60	3.60	127.00	150.00	25.00	72.00	30.00
2	45	F	28.40	10.00	0.70	3.50	158.00	133.00	39.00	92.00	27.00
3	55	F	30.50	8.00	0.80	3.80	179.00	160.00	31.00	116.00	32.00
4	40	F	15.60	6.00	0.50	3.70	176.00	170.00	40.00	102.00	34.00
5	52	F	28.40	14.00	0.70	3.60	141.00	115.00	43.00	75.00	23.00
6	60	F	58.40	15.00	0.60	4.00	171.00	122.00	40.00	107.00	24.00
7	48	F	35.60	18.00	0.50	3.80	173.00	145.00	45.00	99.00	29.00
8	65	F	42.00	20.00	0.80	3.70	156.00	130.00	32.00	98.00	26.00
9	50	F	60.40	22.00	0.90	3.60	186.00	126.00	49.00	112.00	25.00
10	37	F	45.00	14.00	0.70	3.50	171.00	122.00	40.00	107.00	24.00
11	46	F	38.60	10.00	0.60	3.80	172.00	113.00	41.00	108.00	23.00
12	55	F	24.40	8.00	0.50	3.70	145.00	120.00	43.00	78.00	24.00
13	63	F	56.60	12.00	0.80	3.50	195.00	135.00	50.00	118.00	27.00
14	54	F	28.40	10.00	0.70	3.70	165.00	145.00	49.00	87.00	29.00
15	61	F	45.60	11.00	0.60	3.90	183.00	138.00	51.00	114.00	28.00
16	57	F	38.60	15.00	0.70	3.80	148.00	152.00	48.00	69.00	31.00
17	67	F	49.40	13.00	0.80	4.00	159.00	130.00	52.00	81.00	26.00
18	65	F	62.60	18.00	0.80	4.00	179.00	142.00	50.00	119.00	28.00
19	39	F	70.00	20.00	0.90	3.70	188.00	98.00	45.00	115.00	18.00
20	58	F	55.40	14.00	0.50	3.80	193.00	100.00	43.00	130.00	20.00
21	45	M	14.20	9.00	0.70	3.80	156.00	160.00	42.00	82.00	32.00
22	46	M	15.60	14.00	0.60	3.60	150.00	86.00	45.00	46.00	18.00
23	52	M	22.30	15.00	0.80	3.70	148.00	126.00	51.00	50.00	25.00
24	48	M	24.00	8.00	0.70	3.50	145.00	72.00	59.00	72.00	14.00
25	53	M	14.60	10.00	0.50	3.90	161.00	155.00	46.00	84.00	31.00
26	41	M	25.80	12.00	0.40	3.80	186.00	138.00	52.00	106.00	28.00
27	58	M	30.60	15.00	0.50	3.90	172.00	126.00	49.00	98.00	25.00
28	39	M	35.40	8.00	0.80	4.00	160.00	140.00	66.00	66.00	28.00
29	57	M	37.60	10.00	0.60	3.70	138.00	140.00	52.00	58.00	28.00
30	62	M	46.40	15.00	0.70	3.50	152.00	126.00	60.00	67.00	25.00
31	52	M	28.60	12.00	0.60	3.80	145.00	72.00	59.00	72.00	14.00
32	44	M	35.20	10.00	0.70	3.60	150.00	86.00	35.00	97.00	18.00
33	55	M	33.80	8.00	0.80	4.00	148.00	126.00	51.00	72.00	25.00
34	47	M	43.60	16.00	0.90	4.10	167.00	141.00	47.00	92.00	28.00
35	64	M	40.00	12.00	0.60	3.90	171.00	165.00	45.00	93.00	33.00
36	49	M	52.80	13.00	0.50	3.80	154.00	155.00	49.00	74.00	31.00
37	53	M	60.00	15.00	0.60	3.70	200.00	128.00	58.00	120.00	22.00
38	57	M	52.00	23.00	0.60	3.60	175.00	100.00	42.00	113.00	20.00
39	65	M	56.40	14.00	0.70	3.50	142.00	132.00	32.00	74.00	26.00
40	48	M	51.80	10.00	0.70	3.80	173.00	145.00	30.00	113.00	29.00
41	39	M	62.90	8.00	0.80	3.70	181.00	127.00	30.00	125.00	25.00
42	43	M	40.50	4.00	0.80	3.90	190.00	111.00	33.00	135.00	22.00
43	53	M	30.80	6.00	0.70	3.50	171.00	122.00	40.00	107.00	24.00
44	64	M	28.60	5.00	0.60	3.60	188.00	98.00	53.00	115.00	20.00
45	48	M	27.50	17.00	0.50	3.70	159.00	65.00	38.00	108.00	13.00
46	40	M	31.50	14.00	0.70	3.80	176.00	115.00	52.00	101.00	23.00
47	35	M	33.60	8.00	0.80	3.90	194.00	114.00	42.00	130.00	23.00
48	66	M	29.60	12.00	0.70	4.00	138.00	116.00	40.00	75.00	23.00
49	40	M	31.50	10.00	0.90	4.10	173.00	97.00	24.00	130.00	19.00
50	42	M	28.00	13.00	0.80	3.60	178.00	141.00	24.00	126.00	28.00

STANLEY MEDICAL COLLEGE

CHENNAI-600 001

PROFORMA

NAME **:** O.P / I.P.NO **:**

AGE / SEX **:**

RISK FACTORS: HT/ DM / FAMILY H/O DYSLIPIDEMIA /THYROID DYSFUNCTION

DURATION OF CHEST PAIN **:**

INCLUSION CRITERIA: 1) PATIENTS ADMITTED IN ICCU WITH C/O CHEST PAIN
2) ECG SHOWING ST ↓, ST ↑, **T** ↓

EXCLUSION CRITERIA **:** 1) PATIENTS WITH RENAL DISEASE
2) SERUM CREATININE > 3
3) STROKE / CIRRHOSIS

O / E :

PR - BP - RR -

CVS -

RS -

ABD -

CNS -

INVESTIGATION –ALBUMIN - CREATININE-
IMA- CK-MB-
LIPID PROFILE

RESULT :

xg;Gjy; gbtk;

_ _ _ _ _ _ _ _ _ _ _ _ _ _ vd;Dk; ehd;> kUj;Jth; nra;Ak; Ma;Tf;fhf ,uj;jj;ij ghpNrhjid nra;J nfhs;s vd; KO kdJld; rk;kjpf;fpNwd;.

ghpNrhjid gw;wpa tptuq;fis kUj;Jth; $wf;Nfl;L mwpe;J nfhz;Nld;.

,g;gbf;F>

Nehahspapd; cwtpdh;