"HEART-TYPE FATTY-ACID BINDING PROTEIN, IN EARLY DETECTION OF ACUTE MYOCARDIAL INFARCTION – COMPARISON WITH CK – MB, TROPONIN I AND MYOGLOBIN"

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LIST OF ABBREVIATIONS

Abbreviation	Full name
ACS	Acute coronary syndrome
ACC	American College of Cardiology
AMI	Acute myocardial infarction
AUC	Area under curve
α-HBDH	Alpha-hydroxybutyrate dehydrogenase
BIC	Bayesian information criterion
CABG	Coronary artery bypass graft
C-EIA	Competitive enzyme immunoassay
CI	Confidence interval
CK	Creatine kinase
CK-MB	Creatine kinase – MB isoenzyme
CRP	C-reactive protein
cTn	Cardiac troponin
CV	Coefficient of variation
CVD	Cardiovascular disease
DALYs	Disability-adjusted life-years
ECG	Electrocardiography
ED	Emergency department
EDTA	Ethylene diamine tetra acetic acid
eGFR	Estimated glomerular filtration rate

ELISA	Enzyme-linked immunosorbent assay
ES	Emergency service
ESC	European Society of Cardiology
ESR	Erythrocyte sedimentation rate
FFAu	Unbound free fatty acids
GP-BB	Glycogen phosphorylase – BB isoenzyme
HDL	High-density lipoprotein
H-FABP	Heart-type fatty acid binding protein
HICs	High-income countries
HR	Hazard ratio
Hs-cTn	High sensitive cardiac troponin
ICCU	Intensive cardiac care unit
ICD	Implantable cordioverter defibrillator
IHD	Ischemic heart disease
LBBB	Left bundle branch block
LCFA	Long chain fatty acid
LDL	Low-density lipoprotein
LMICs	Low- and middle-income countries
LOB	Limit of blank
LOD	Limit of detection
LOQ	Limit of quantitation
LR	Likelihood ratio
LTIA	Latex turbidimetric immunoassay

LVH	Left ventricular hypertrophy
NACB	National Academy of Clinical Biochemistry
NCCP	Non-cardiac chest pain
NIH	National Institutes of Health
NPV	Negative predictive value
NSTE-ACS	Non-ST segment elevation acute coronary syndrome
NSTEMI	Non-ST segment elevation myocardial infarction
OPD	Out patient department
PE	Pulmonary embolism
PI	Isoelectric point
PPV	Positive predictive value
PTCA	Percutaneous Transluminal Coronary Angioplasty
PTP	Pretest probability
RIA	Radio-immuno assay
RLUs	Relative light units
ROC curve	Receiver operating characteristic curve
RR	Relative risk
RV	Reaction vessel
SAR	South asian region
SD	Standard deviation
STEMI	ST segment elevation myocardial infarction
UA	Unstable angina
WHO	World health organization

ABSTRACT

Background: Early diagnosis and therapeutic intervention can improve the outcome of acute myocardial infarction (AMI). However, there are no satisfactory cardiac biomarkers for the diagnosis of AMI within 6 hours of onset of symptoms. Among novel biochemical markers of AMI, heart-type fatty acid binding protein (H-FABP) is of particular interest.

Aim: The study aimed to investigate whether H-FABP measurement provides additional diagnostic value to that of conventional cardiac markers in AMI within first 6 hours after the onset of symptoms.

Materials and methods: A total of 120 patients presenting with acute chest pain within 6 hours of onset, suggestive of AMI were included in the present study according to the inclusion and exclusion criteria. In all the cases and controls, complete clinical history, ECG, echocardiography and other routine investigation findings were noted. Serum H-FABP concentration was measured by immunoturbidimetric method, serum troponin I and myoglobin concentrations were measured by chemiluminescence immunoassay and serum CK-MB activity was estimated by immuno-inhibition method. Diagnosis of AMI was done according to the European Society of Cardiology/ American College of Cardiology Committee (ESC/ACCC) Criteria. 60 cases were diagnosed as having AMI cases and remaining 60 cases were age and sex matched non-cardiac chest pain controls. The cases and controls were further divided into 2 subgroups depending on the time since onset of chest pain as those subjects within 3 hours and those between 3-6 hours of onset of chest pain.

Statistical Analysis: Data was presented as mean ± SD values. Differences between means of two groups were assessed by Student t-test. Sensitivity, Specificity, Positive

predictive value, Negative predictive values were calculated and ROC curve analysis was done to assess the diagnostic validity of each study parameter.

Results: The serum mean levels of H-FABP, CK-MB, troponin I, and myoglobin were significantly higher ($p > 0.01$) in AMI cases when compared to that of non-AMI controls in both 0 – 3 hrs (40.4 ± 56.3, 26.2 ± 19.2, 5.9 ± 16.2, 364.9 ± 363.9 vs 4.7 ± 7.3, 18.6 ± 9.5, 0.0048 ± 0.0077, 55.7 ± 53.4 respectively) and 3 – 6 hrs (76.3 ± 56.1, 36.9 ± 48.9, 8.5 ± 16.9, 584.5 ± 393.9 vs 6.7 ± 8.6, 25 ± 10.1, 0.0087 ± 0.0079, 90.8 ± 74.2 respectively) groups. The sensitivity, specificity, positive and negative predictive values of H-FABP (92.3%, 88.5%, 88.9%, 92.0% in 0-3 hr group; 94.1%, 79.4%, 82.1%, 93.1% in 3-6 hr group, respectively) were significantly greater than CK-MB (23.1%, 61.5%, 37.5%, 44.5% in 0-3 hr group; 52.9%, 58.8%, 56.3%, 55.6% in 3-6 hrs group, respectively) and myoglobin (73.1%, 76.9%, 76.0%, 74.1% in 0-3 hrs group; 88.2%, 70.6%, 75.0%, 85.7% in 3-6 hrs group, respectively) but were lesser than Troponin I (96.2%, 100%, 100%, 96.3% in 0-3 hrs group; 100%, 100%, 100%, 100% in 3-6 hrs group, respectively) in patients with suspected AMI in both within 3 hours and 3 – 6 hours groups. Receiver Operating Characteristic (ROC) curves demonstrated greatest diagnostic ability for Troponin I (AUC = 0.997 & P = 0.000 in 0-3 hr group; AUC = 0.982 & P = 0.000 in 3-6 hrs group) followed by H-FABP (AUC = 0.886 & P = 0.000 in 0-3 hrs group; AUC = 0.911 & P = 0.000 in 3-6 hrs group), myoglobin (AUC = 0.841 & P = 0.001 in 0-3 hrs group; AUC = 0.860 & P = 0.000 in 3-6 hrs group) and CK-MB (AUC = 0.640 & P = 0.163 in 0-3 hrs group; AUC = 0.616 & P = 0.177 in 3-6 hrs group) within 3 hrs and 3 – 6 hrs after the onset of chest pain.

Conclusion: The diagnostic value of H-FABP is greater than CK-MB and myoglobin but slightly lesser than troponin I for the early diagnosis of AMI within first 6 hours of chest pain. H-FABP can be used as an additional diagnostic tool for the early diagnosis of AMI along with troponin I.

Key words: Myocardial infarction**;** Heart-type fatty acid binding protein, human; Troponin-I; Myoglobin; Creatine Kinase, MB; Immunoturbidimetric method; ROC analysis.

Chapter 1
Introduction

INTRODUCTION

1.1 Purpose of the study

Chest pain is the second most common and important medical complaints, in the patients attending the emergency department, constituting 5% of all the complaints.[1] But only 15-25% of patients with acute chest pain will actually have ischemic heart disease (IHD), after a thorough diagnostic evaluation. Gastro-oesophageal diseases, pulmonary and musculo-skeletal disorders are the other common causes of chest pain. Differentiating IHD patients and/or other life threatening disorders from those with noncardiovascular and/or non–life threatening chest pain is the real challenge in the emergency department. There are many uncertainties and legal aspects, which makes the decision difficult. An early and accurate diagnosis of chest pain may be of great help for the clinician to avoid unnecessary admissions or inappropriate discharge.[2]

Ischemic heart disease (IHD) can be defined as "group of heart conditions in which there is an inadequate supply of blood and oxygen to a portion of the myocardium, which typically results when there is an imbalance between myocardial oxygen supply and demand". Most commonly IHD is because of atherosclerotic disease involving one or more epicardial coronary arteries sufficient to cause a regional decrease in myocardial blood supply and inadequate oxygen perfusion of the myocardium supplied by the involved coronary artery. IHD comprises "acute myocardial infarction (AMI), where blood supply to a part of the myocardium is blocked for long enough that part of the heart muscle undergoes ischemic damage and eventually dies if untreated".[3]

Cardiovascular disease (CVD) has emerged as the single most important cause of death worldwide during the last decade. Presently, CVD is the most common cause of the death in all low- and middle-income countries. In sub-Saharan Africa, CVD is the leading cause of death among persons older than 45 years of age. Between 1990 and 2010, deaths from CVD increased from 26% to 29.5% of all deaths globally—a reflection of the rapidity of the epidemiologic transition—particularly in low- and middle-income regions. Many studies in India and Pakistan suggest substantial morbidity and mortality resulting from IHD in this region. In 1990, 1.18 million people died in India as a consequence of IHD; by 2010, this number increased to an estimated 2.03 million. CVD probably represents 25% of all deaths in India. Studies also show that IHD prevalence is higher in men and in urban residents. Prevalence of IHD in India recently was estimated at more than 10% in urban areas and 4.5% in rural areas.[4] In light of the projection of large increases in IHD throughout the world, IHD is likely to become the most common cause of death worldwide by 2020.[3]

The early and accurate diagnosis and treatment of AMI is most important to prevent the serious complications and mortality. Mortality can be reduced from 9% to 3% if AMI is diagnosed, and treated properly within one hour after the onset of symptoms (so called ‘golden hour’), and the risk of death is 5 times higher if the treatment is delayed for 3 – 4 hrs. Unfortunately, at least one fifth of the AMI cases go undetected, either because of the atypical signs and symptoms or unclear electrocardiographic (ECG) changes and/or delayed elevation of cardiac biomarkers in the blood. Additionally, almost 40% of patients presenting with acute chest pain can be saved from the complications and unnecessary hospital expenses by accurately precluding the AMI.[5]

The diagnosis of AMI is traditionally dependent upon a combination of ischemic symptoms, ECG changes and elevation in serum biomarkers. However, the symptoms are often rather atypical or absent, and around 33% of the patients arriving at the emergency department with MI may not have chest pain. Similarly, ECG changes that aid with early diagnosis may be nonspecific or even absent in around 40% of the patients. Currently used cardiac biomarkers such as cardiac troponins, and creatine kinase – MB (CK-MB), although more specific for myocardial damage, have low sensitivity in the early period, whereas myoglobin level increase early in the blood (within 2 hours after myocardial infarction), but lacks cardiac specificity. In this way, their utilization in the determination of AMI in the early hours (within 6 hrs) is restricted. [6]

Among the novel and most promising cardiac biomarkers for the early and accurate diagnosis of AMI, "heart-type fatty acid binding protein" (H-FABP) is of particular interest. H-FABP is a small soluble protein, consisting of 132 amino acids with a molecular weight of 15 kDa. It is one of the most abundant proteins in the cardiac muscle, which constitutes 5 – 15% of the total cytosolic protein pool inside the myocyte. H-FABP is relatively specific for heart, because of its much low concentration in other tissues. It's primary function is to transport intracytoplasmic fatty acids in to the mitochondria for oxidation. Several earlier researchers have found that, diagnostic value of H-FABP might be greater than currently available conventional cardiac markers for the early diagnosis of AMI. However, there is still a significant uncertainty with regard to the utilization of H-FABP in the early detection of AMI. Serum H-FABP estimation is restricted to the clinical research due to the absence of quick and simple technique for its

measurement. However recently, a novel automated ‘immuno-turbidimetric method’ for serum H-FABP estimation has been developed.[7]

Hence, the aim of the present study was to evaluate whether serum H-FABP measurement within 6 hours of onset of symptoms provides additional diagnostic value to that of conventional cardiac markers in acute myocardial infarction. Objective of the study was to compare the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic ability of H-FABP with the conventional cardiac biomarkers such as CK-MB, troponin I and myoglobin in patients presenting with chest pain between 0 – 3 hrs and 3 – 6 hrs after the onset of chest pain.

Chapter 2
Aims & Objectives

AIMS AND OBJECTIVES

2.1 Aim:

1. To evaluate the diagnostic utility of H-FABP in comparison with CK-MB, troponin I and myoglobin in the early accurate detection of acute myocardial infarction.

2.2 Objectives:

1. To compare the sensitivity, specificity, positive predictive value, negative predictive values of H-FABP with the conventional cardiac biomarkers (CK-MB, Troponin I and Myoglobin) in suspected patients of acute myocardial infarction presenting with chest pain within 3 hrs and 3 – 6 hrs after the onset of chest pain.
2. To compare the diagnostic ability of H-FABP with the conventional cardiac biomarkers (CK-MB, Troponin I and Myoglobin) in suspected patients of acute myocardial infarction presenting with chest pain within 3 hrs and 3 – 6 hrs after the onset of chest pain.

Chapter 3
Review of Literature

REVIEW OF LITERATURE

3.1 Basic anatomy of heart

General organization

The heart is an organ made up of a pair of valved muscular pumps. These two muscular pumps (the 'right and left' hearts) are 'physiologically separate', even though the fibromuscular framework and conducting tissues of these pumps are structurally interwoven.

The right heart includes right atrium and right ventricle. The right atrium receives venous blood from the whole body through the 'superior and inferior venae cavae', along with the venous blood from the heart itself via the 'coronary sinus'. Right atrium further pumps blood in to the right ventricle through the right atrioventricular opening with tricuspid valve. Right ventricle pumps blood in to the lungs through the pulmonary trunk and pulmonary arteries.

Left atrium together with left ventricle forms the left heart. Left atrium receives all the oxygenated blood from the lungs through the pulmonary veins and some coronary venous blood, and pumps it in to the left ventricle through the left atrioventricular opening and mitral valve. Ventricular contraction closes the mitral valve, and opens the aortic valve, pumping the blood into the 'ascending aorta', and hence supplying the oxygenated blood to the entire body, including the heart itself.[8]

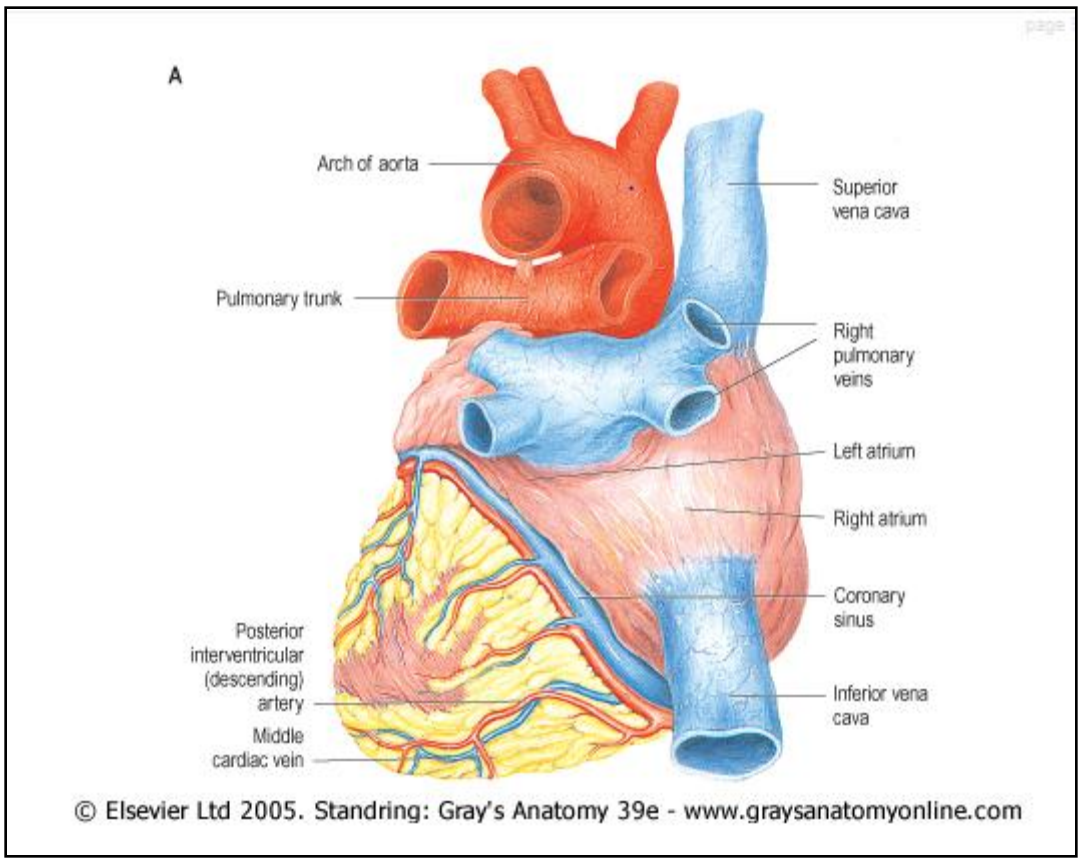


Figure – 1: The human heart: General organization[8]

Size, shape and external features of heart

The heart is a conical / pyramid shaped, hollow and fibromuscular organ. It has a base, apex and various surfaces and borders. It is located in the middle mediastinum and is covered by pericardium. The heart is obliquely located, behind the body of the sternum and the adjoining parts of costal cartilages and ribs, such that, 1/3rd of it lies to the right and 2/3rd to the left of the median plane. An average adult heart measures 12 cm x 9 cm x 6 cm, and weighs around 300 g in males and 250 g in females.[8]

Vascular supply of the heart

The right and left coronary arteries issue from the ascending aorta (**Fig - 2**).

I] Right coronary artery: It is a branch of anterior aortic sinus, which inturn arises from ascending aorta. Its branches can be divided into 2 groups as, those arising out of first part (upto inferior border) of the artery, and those arising from the second part (upto the crux).

Branches from the first segment

1. Ventricular rami
    a. "Right conus artery" – Supplies the infundibulum of the right ventricle. Sometimes it directly arises from the anterior aortic sinus. In such case it is called the "*third coronary artery*".
    b. "Right anterior ventricular rami" – They supply the sternocostal wall of the right ventricle. These are 3 or 4 in number.
    c. "Right marginal artery" – It supplies the adjoining surfaces of right ventricle by running through the inferior border of the heart towards the apex.
2. Atrial rami –
    a. These can be grouped in to anterior, lateral and posterior rami. They supply the right atrium.
    b. Sinu-atrial nodal artery – It arises in 65% of the cases from the first segment of right corornary artery and in 35% of subjects from circumflex branch of left coronary artery.

Branches from the second segment

1. "Right posterior ventricular rami" – They supply the diaphragmatic surface of the right ventricle.
2. "Posterior interventricular branch" – Provides few branches to the diaphragmatic surface of the right and left ventricles. Most significant branches are septal rami which supply the postero-inferior one-third of the ventricular septum. First septal ramus of the posterior interventricular branch supplies the atrio-ventricular node.
3. "Right posterior atrial rami" – Supply the posterior surface of both right and left atria.

II] Left coronary artery – arises from the left posterior aortic sinus of the ascending aorta. It usually divides into 2 branches

1. Anterior interventricular artery – It is a continuation of the left coronary artery. Its branches are
    a. Anterior ventricular rami: left ventricle (most of the portion) and a small strip of right ventricle are supplied by these branches. One of the right anterior ventricular rami forms the left conus artery which supplies the infundibulum of right ventricle.
    b. Septal rami – Supply the antero-superior two-third of the ventricular septum.
2. Circumflex artery –
    a. Atrial and ventricular rami supply the adjoining surfaces of left atrium and left ventricle.
    b. Sinu-atrial nodal artery – Arises from circumflex branch in 35% of the subjects

c. Left margibal artery

d. Posterior interventricular artery extends as a continuation of circumflex branch in 10 – 20% of the individuals.

e. Atrial branch[9]

Summary of coronary distribution

Most commonly, the right coronary artery supplies all the right ventricle (except a small region right of the anterior interventricular groove), a variable part of the left ventricular diaphragmatic aspect, the posteroinferior one-third of the intraventricular septum, the right atrium and part of the left, and the conducting system as far as the proximal parts of the right and left crura. Left coronary distribution is reciprocal, and includes most of the left ventricle, a narrow strip of right ventricle, the anterior two-thirds of the interventricular septum and most of the left atrium.[9]

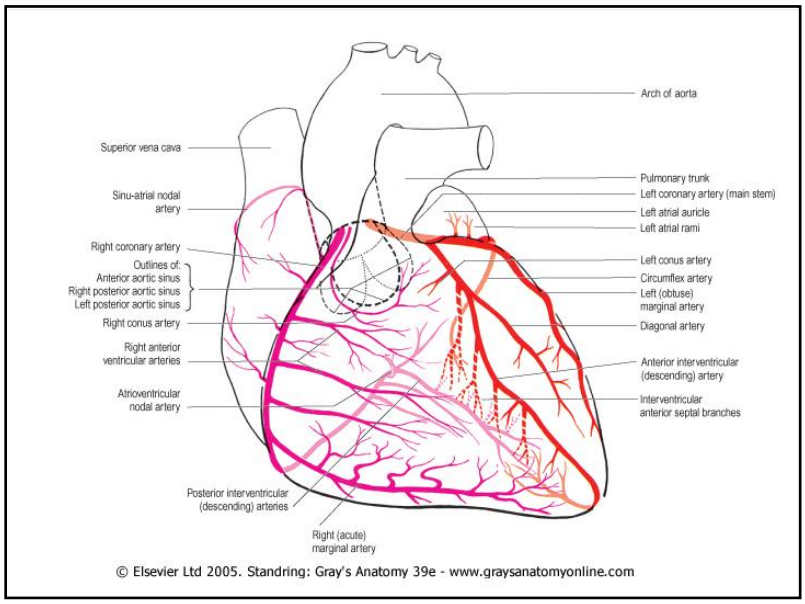


Figure – 2: Vascular supply of heart[9]

Cardiac veins

Coronary sinus and its branches, along with the anterior and small cardiac veins drain the deoxygenated blood from the heart, ultimately draining in to the right atrium.[8]

Lymphatic drainage of the heart

Lymphatic vessels from the heart form subepicardial, myocardial and subendocardial plexuses. The subendocardial, myocardial plexuses drain into the subepicardial plexuses. Subepicardial plexuses drain in to the left and right cardiac collecting trunks. Afferent lymphatic vessels from the right atrium, and right border and diaphragmatic surface of the right ventricle drain in to the right cardiac collecting trunk, which end in a brachiocephalic node, usually on the left. 2 - 3 left cardiac collecting trunks terminate in an inferior tracheobronchial node.[8]

Nerve supply of the heart

"Sympathetic as well as parasympathetic nerve fibres innervate the heart. The muscle fibres of the atria and ventricles and the cardiac electrical conducting system are supplied by adrenergic nerves from the cervical sympathetic chain. Positive inotropic and chronotropic effects are caused by β_1-adrenergic receptors, whereas β_2- adrenergic receptors predominate in cardiac vascular smooth muscle and cause vasodilatation. Vagus nerve supplies parasympathetic pre-ganglionic fibres and sensory fibres to the heart. AV and SA nodes are supplied by the cholinergic nerves via muscarinic (M2) receptors. Heart rate is slow under resting conditions, as the vagal inhibitory activity predominates. During the exercise, emotional stress, fever, etc, the heart rate increases because of the associated adrenergic stimulation".[10]

3.2 Cardiovascular physiology

Cardiac Cycle

The two phases of cardiac cycle are known as diastole (period of relaxation) and systole (period of contraction). Diastole is the period during which, oxygenated blood enters the left atrium, from the lungs, via pulmonary veins, and deoxygenated blood from other parts of the body returns to the right atrium via superior and inferior vena cava. Passive filling of the ventricle also occurs during this period as the AV valves are open. At the end of diastole, as the atria contract, remaining blood is forced through the AV valves into the respective ventricles. During the period of systole, contraction of the ventricles closes the AV valves, as ventricular pressure exceeds the atrial pressure, and the pulmonary and aortic valves are opened when ventricular pressure exceeds pressure in the pulmonary arteries and the aorta, and blood flows into those conduits. During systole, normal blood pressure in the aorta is typically 120 mm Hg and during diastole, it falls to about 70 mm Hg. Under resting conditions, the heart pumps between 60 and 80 times per minute. Stroke volume (i.e., the volume of blood pumped by the left ventricle with each contraction) is roughly 50 mL, so the cardiac output (the amount of blood pumped out of the heart per minute) is roughly 3 L/minute.[11]

Cardiac Conducting System

The cardiac cycle is tightly regulated by the cardiac electrical conducting system. Specialized cardiac conducting system generates electrical impulses and carries them to the myocardium. The electrocardiogram (ECG) is a graphical recording of the changes in electrical potential caused by excitation of the heart muscle and it is detected at the body surface. Clinically, the ECG is helpful in detecting “anatomic, metabolic, ionic, and

hemodynamic changes" in the heart. A large number of anatomic and physiologic changes and the clinical situation itself influence the clinical sensitivity and specificity of ECG abnormalities. Under normal resting conditions, cardiac cycles are similar and each cycle includes three major components (Figure – 3) namely, atrial depolarization (P wave), ventricular depolarization (QRS complex), and repolarization (ST segment and T wave). P wave in the ECG representing atrial depolarization, produces contraction of the atria. Ventricular depolarization, depicted by the QRS complex, produces ventricular contraction. Electrical recovery of the ventricles produces the ST segment and the T wave.

A routine ECG is recorded using 12 leads. Six of them are called limb leads (I, II, III, aVR, aVL, and aVF), because they are recorded between arm and leg electrodes; another six are recorded across the sternum and left precordium and hence are called precordial or chest leads (V1, V2, V3, V4, V5, and V6). Each lead records the same electrical impulse but in a different position relative to the heart.[11]

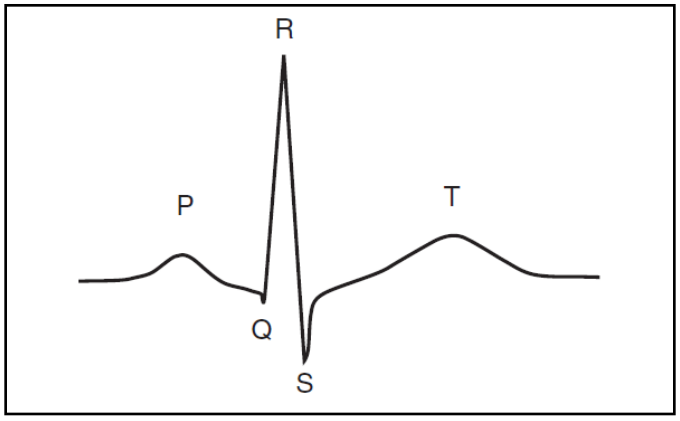


Figure - 3: Electrocardiogram, serial tracing of a normal single heartbeat. Each heart beat produces five major waves: P, Q, R, S, and T. [11]

3.3 Cellular basis of cardiac contraction

Cardiac ultrastructure

Cardiac muscle is an involuntary, striated muscle made of *cardiac muscle fibres*. The fibres are branched and interdigitate freely with each other, having 17–25 μm diameter and 60–140 μm length. The muscle fibres are made up of many fibrils called myofibrils, which lie parallel to one another and are striated. They have sarcoplasmic reticulum with plenty of cytoplasm, mitochondria and glycogen, surrounded by sarcolemma. They are composed of serially repeating structures called the *sarcomeres.*

The characteristic striations are due to the alternate dark and light cross bands. The dark band present in the centre of sarcomere is called as 'A' band, which is of constant length (1.5 μm). In the centre of 'A' band is 'H' band of 0.5 μm length. The light bands of variable length present on either side of the 'A' band are called 'I' bands. The narrow lines present in the centre of each 'I' band are called 'Z' lines. Sarcomere is the contractile unit of the muscle present between two adjacent 'Z' lines. The distance between the two adjacent Z lines changes with each contraction and relaxation and it ranges between 1.6 and 2.2 μm.[12]

The Contractile Process

The sliding filament theory:

It is the process in which the shortening of the contractile elements in the muscle occurs, which is brought about by the sliding of actin filament over the myosin filaments. In a resting muscle, no cross bridges are formed since troponin I is loosely bound to actin

and tropomyosin covers the actin sites where myosin heads bind to actin. During the activation of cardiac myocytes, the action potential releases ca^{2+} from the terminal cisterns. Binding of calcium to troponin C, results in conformational change in tropomyosin, uncovering the seven myosin binding sites on actin filaments. The heads of the myosin slide over the actin at 90 degree angle, resulting in muscle shortening. The myosin dissociates from actin with the breakdown of ATP. Repetitive interaction between myosin heads and actin filaments is termed cross-bridge cycling. The width of 'A' bands is constant, whereas the 'Z' lines move closer when the muscle contracts and farther apart when it is stretched. Intra-cytoplasmic Ca^{2+} level determines the inotropic state of the heart.[12] (Figure – 4)

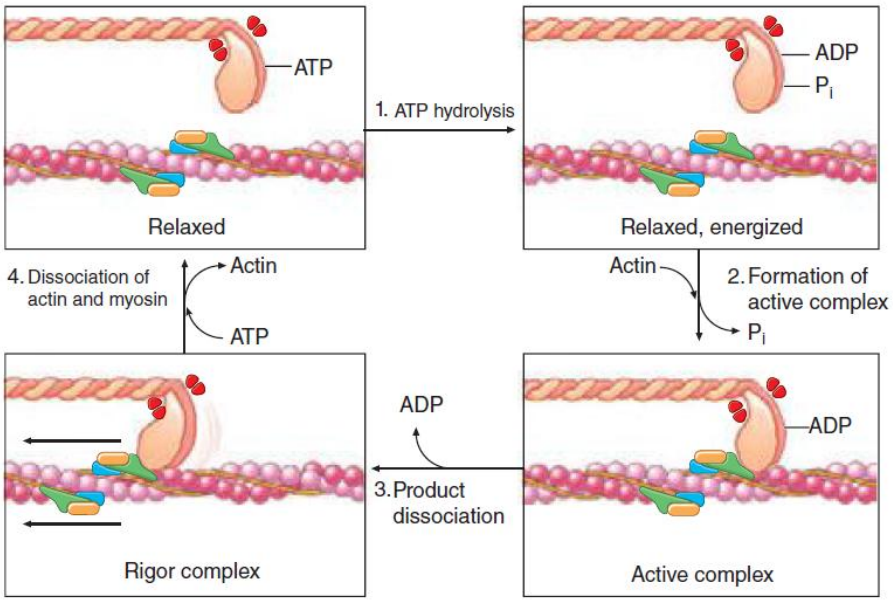


Figure – 4: Four steps in cardiac muscle contraction and relaxation.[12]

3.4 Approach to the patient with chest pain

One of the most common medical complaints among the patients in the 'emergency department' (ED) is 'acute chest pain' which accounts for around 8 million hospital visits in the United States per year. Although acute coronary syndrome manifests most often as chest pain, just 15 - 25% of patients presenting with acute chest pain will really have ACS, after the proper diagnostic evaluation. Unfortunately, in around 2% of chest pain patients, the diagnosis of ACS is missed, which can lead to adverse consequences. The short-term mortality in patients with acute myocardial infarction (AMI) who are mistakenly discharged from the ED is twofold high when compared to those patients who are diagnosed and treated properly. For patients with a lower risk for complications, however, these concerns must be balanced against the cost and inconvenience of admission and against the risk for complications from tests and procedures with a low probability of improving patient outcomes. Hence, the real challenge in the emergency department is to differentiate chest pain patients with life threatening conditions including ACS from those with non–life threatening and / or non-cardiovascular chest pain.[2]

Common causes of acute chest pain

Table – 1: Common causes of acute chest pain[2]

SI. No.	System	Syndrome / Disease
01.	Cardiac	Stable Angina Unstable angina

		Acute myocardial infarction Pericarditis
02.	Vascular	Aortic dissection Pulmonary embolism Pulmonary hypertension
03.	Pulmonary	Pleuritis / Pneumonia Trachea-bronchitis Spontaneous pneumothorax
04.	Gastro-intestinal	Gastro- Esophageal reflux disease Peptic ulcer Gall bladder disease Pancreatic disorder
05.	Musculoskeletal	Costochondritis Cervical disk disease Trauma / strain
06.	Infectious	Herpes zoster
07.	Psychological	Panic disorder

3.5 Coronary heart disease / Ischemic heart disease (CHD / IHD)

3.5.1 Definition

'Ischemic heart disease' (IHD) can be defined as "condition in which there is an inadequate supply of blood and oxygen to a portion of the myocardial tissue, which

typically occurs when there is an imbalance between myocardial oxygen supply and demand. The most common cause of myocardial ischemia is atherosclerotic disease of one or more epicardial coronary arteries sufficient to cause a regional reduction in myocardial blood flow and inadequate perfusion of the myocardium supplied by the involved coronary artery".[3]

3.5.2 Global burden of coronary heart disease

Over the past decade, cardiovascular disease (CVD) is seen as the single most important cause of death globally. In the year 2010, an estimated 16 million deaths occurred and 293 million disability-adjusted life-years (DALYs) were lost due to CVD, which represents around 30 percent of all deaths and 11 percent of all DALYs lost in that particular year. Like many developed countries in the previous century, now developing countries are encountering an alarming increase in the rate of CVD incidence.

In all low- and middle-income regions, CVD is the most common cause of deaths, with the exception of sub-Saharan Africa, where it is the leading cause of death among persons older than 45 years of age. Between 1990 and 2010, deaths from CVD increased from 26% to 29.5% of all deaths globally, which shows that, epidemiologic transition is occurring rapidly, especially in low- and middle-income regions.

The six low-income and middle-income countries as characterized by World Bank show a huge difference in the CVD burden. Death rates due to CVD are 60%, 10% and 36% in middle income (Eastern Europe), low income (sub-Saharan Africa) and high income countries respectively. The overall rise in the global burden of CVD and the distinct regional patterns of distribution are in part because of the epidemiologic

transition, which includes four basic stages: pestilence and famine, receding pandemics, degenerative and man-made diseases, and delayed degenerative diseases. Progression through these stages has dramatically shifted the predominant causes of death over the past two centuries, from infectious diseases and malnutrition in the first stage to CVD and cancer in the third and fourth stages.[4]

Current variations in the global burden of cardiovascular disease

Global CVD rates are largely driven by the CVD rates in low income countries, because 85% of the world's population lives in these countries. Although the CVD rates fall in high income countries, CVD rates worldwide are increasing, Worldwide, the number of CVD deaths increased by 31% between 1990 and 2010, but age-adjusted death rates decreased by 21.2% in the same period, suggesting significant delays in age at occurrence and/or improvements in case-fatality rates.[4]

South Asia

CVD accounts for 20% of all deaths in the SAR. In the year 2010, CHD was the leading cause of mortality—responsible for 10.6% of reported fatalities, or 1.8 million deaths, and more than half of CVD deaths. Nearly 60.5 million DALYs are lost due to CVD in the SAR, accounting for 10% of all DALYs lost.

CHD is responsible for 4.6% of the DALYs lost because of CVD. Mortality rates for CVD are increasing in the region. Several earlier studies in India and Pakistan have found substantial morbidity and mortality resulting from CHD in this region. In 1990, 1.18 million people died in India as a consequence of CHD; by 2010, this number increased to an estimated 2.03 million. CVD probably represents 25% of all deaths in

India. Studies also show that CHD prevalence is higher in men and in urban residents. Prevalence of CHD in India recently was estimated at more than 10% in urban areas and 4.5% in rural areas.

Another demographic trend in the SAR is a considerable increase in urban residents, a shift that usually correlates with increased CHD rates. Currently, 31% of all inhabitants in the SAR live in urban areas, a number that is expected to rise further. A review of epidemiologic studies in the country found that between 1965 and 2005, CHD prevalence increased from approximately 4% to 12% in urban populations. Rural populations are experiencing similar increases in CHD prevalence. More recent studies in the rural region of Andhra Pradesh in South India have found higher prevalence of CHD in many rural areas. In the same studies, CHD death rates were found to be more than 15%, suggesting that there is no rural-versus-urban protection factor—or that the urban CHD death rates could be much higher, if measured more carefully.

In India, the increase in mortality due to CHD contributes to the economic burden of the country. Data indicate that symptoms of CHD appear 5 to 10 years earlier in this region when compared to Western European and Latin American countries. Furthermore, CVD affects a substantial proportion of working-age citizens. A study in rural India, for example, found that 51% of all CVD deaths occurred in individuals younger than 70 years of age.[4]

3.5.3 Patho-physiology

The concept of myocardial blood supply and demand balance is crucial for understanding the ‘pathogenesis of myocardial ischemia’. Under normal circumstances,

the myocardium is capable of controlling the oxygen-rich blood supply for any given level of oxygen demand so as to prevent the myocardial ischemia and infarction. The myocardial contractility and it's wall tension, and heart rate are the major factors influencing the myocardial oxygen demand. Adequate coronary blood supply and sufficient oxygen-carrying capacity of the blood, normal pulmonary function, and hemoglobin concentration are essential for the adequate supply of nutrients and oxygen to the myocardium. The coronary blood supply occurs in a phasic manner. Major amount of coronary blood flow occurs during the diastole of the cardiac cycle.

Normally, the coronary blood vessels have a great capacity to vary their resistance and hence the blood flow, by the virtue of dilation, when the need arises. For example, 'Metabolic regulation' can be seen during severe exercise and emotional stress, etc. when the myocardial oxygen demand increases, and the coronary vascular resistance decreases, thereby supplying the adequate oxygen and nutrients. Also, Autoregulation occurs by the adaptation of coronary resistance vessels whenever there is alteration in the blood pressure so as to meet the myocardial oxygen demand.

Atherosclerosis, by narrowing the coronary arteries, limits the proportionate increase in the myocardial blood supply whenever there is increase in the myocardial oxygen demand as it occurs in severe exercise and / or emotional stress. When there is a severe reduction in coronary artery luminal diameter, the basal level of myocardial blood flow is decreased. Rarely, myocardial blood supply is also reduced in cases other than atherosclerosis, such as coronary artery spasm (Prinzmetal's angina), coronary thrombi and emboli, and coronary ostial stenosis in aortitis. Structural anomalies of coronary

vasculature may cause ischemic manifestations in infants, but in adults this is very rare, Ex: "left anterior descending coronary artery arising from the pulmonary artery".

"Myocardial ischemia" can also occur in severe 'left ventricular hypertrophy' due to aortic stenosis where myocardial oxygen demand is markedly increased. Extremely severe anemia or carboxyhemoglobinemia can rarely cause myocardial ischemia because of decreased oxygen carrying capacity of the blood. Not infrequently, more than one causes of ischemia can be present simultaneously in a patient, such as 'left ventricular hypertrophy due to hypertension' leading to an increase in oxygen demand and a decrease in myocardial blood supply due to 'coronary atherosclerosis and/or severe anemia'.[3]

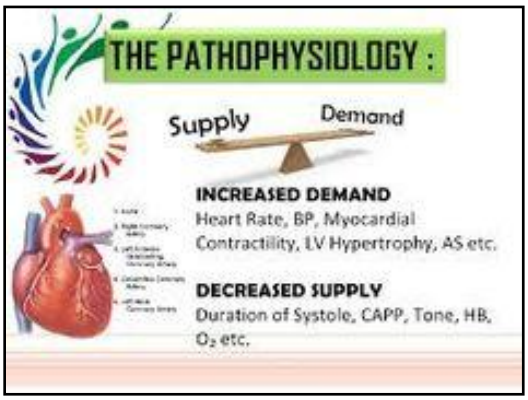


Figure – 5: Pathophysiology of ischemic heart disease: Role of myocardial oxygen supply and demand balance[3]

Atherosclerosis

Epicardial coronary arteries are the common sites for atherosclerosis. The normal functions of vascular endothelium are "local regulation of vascular tone, maintenance of an antithrombotic endothelial surface, and regulation of inflammatory cell adhesion and

diapedesis". The risk factors of atherosclerosis such as "high plasma levels of low density lipoprotein (LDL), low plasma high density lipoprotein (HDL), cigarette smoking, hypertension, and diabetes mellitus" interfere with the normal functioning of the vascular endothelium leading to constriction of vessel, thrombus formation, and abnormal interactions between activated vascular endothelium and blood cells, especially monocytes and platelets. These functional changes in the blood vessels ultimately lead to the "deposition of fat, smooth muscle cells, fibroblasts, and intercellular matrix" in the subintimal space leading to the formation of atherosclerotic plaque.

Atherosclerosis develops at irregular rates at different segments of the epicardial coronary arteries, eventually leading to plaque formation and segmental reductions in luminal diameter. When the diameter of an epicardial artery is reduced to 50%, there is a decrease in the capacity of coronary resistance vessels, to increase blood flow during the situations of increased myocardial oxygen demand. When the coronary artery lumen is obstructed by around 80%, myocardial blood flow at rest also may be insufficient. Further, even a little reduction in the previously narrowed vascular lumen can markedly decrease coronary blood flow, resulting in 'myocardial ischemia at rest or with minimal stress'.

Erosion of cap and rupture of an 'atherosclerotic plaque' exposes its contents to blood leading to the activation and aggregation of platelets, and activation of the coagulation cascade, which further causes deposition of fibrin strands. Thus a 'thrombus' is formed, consisting of platelet aggregates and fibrin strands, which can also trap RBCs and can further narrow the coronary lumen, causing 'myocardial ischemia'.

The quantity of myocardial tissue undergoing ischemia and the severity of clinical manifestations are determined by the location of the coronary obstruction. Thus, "obstructions in the left main coronary artery and the proximal left anterior descending coronary artery, are particularly hazardous". "Chronic coronary artery narrowing, especially when it develops gradually is frequently accompanied by the development of collateral vessels. When such collaterals are well developed, can by themselves provide sufficient blood flow to sustain the viability of the myocardium at rest but not during conditions of increased demand".[3]

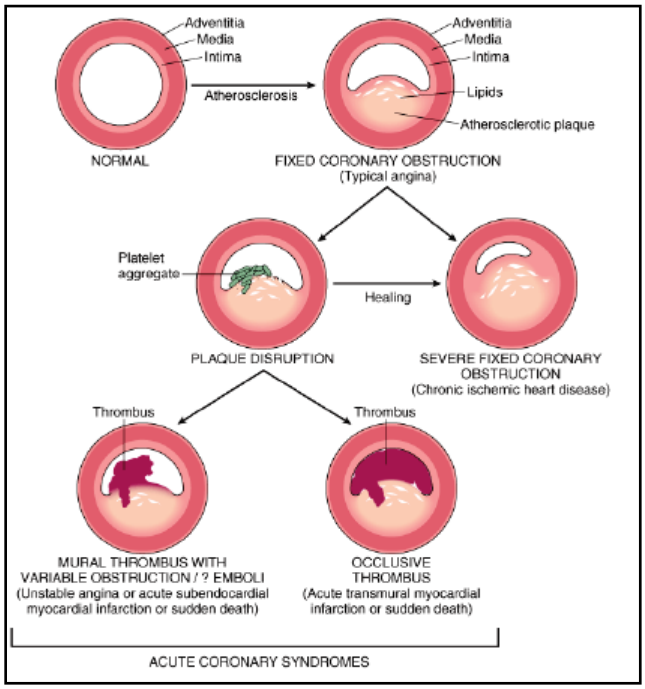


Figure – 6: Pathophysiology of ischemic heart disease: Atherosclerosis[13]

3.5.4 Effect of ischemia

During the episodes of insufficient blood supply to myocardium due to coronary atherosclerosis, myocardial tissue oxygen concentration decreases which can disrupt the

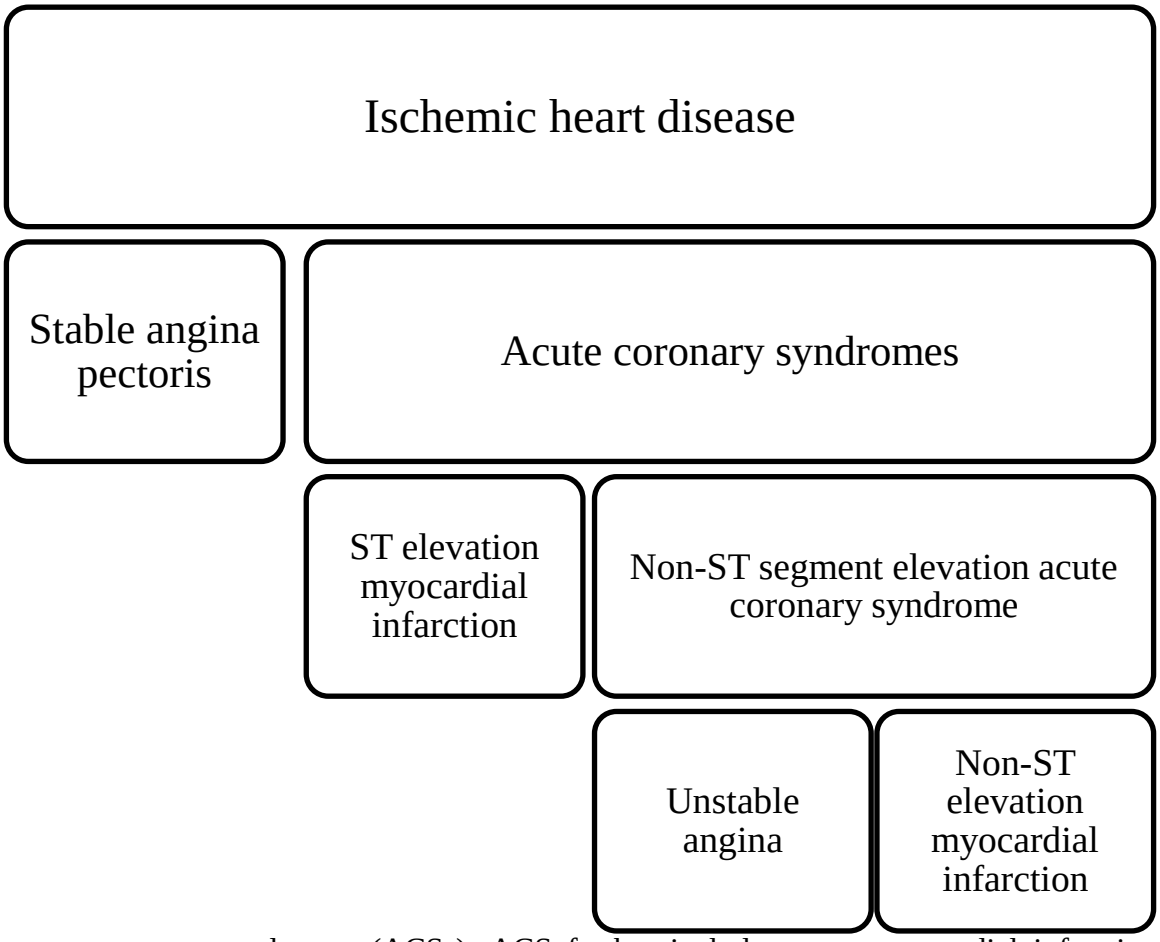
biochemical, mechanical, and electrical functions of the myocardium, adversely affecting the myocardial contractility. Impaired ventricular contractility at places affected by myocardial ischemia can cause "segmental hypokinesia, akinesia, or, in severe cases, bulging and dyskinesia", which in turn can decrease the myocardial contractility and cardiac output. Ischemia because of the sudden, total or partial obstruction of coronary artery can lead to "failure of normal cardiac relaxation and then contraction". The poor blood supply to the subendocardial portion of the heart causes more severe form of ischemia when compared with the ischemia of subepicardial region. Sudden left ventricular failure can occur when ischemia involves large portions of the ventricle, and ischemia of the papillary muscles can result in mitral regurgitation. When ischemia lasts for a shorter period of time, it causes "angina pectoris" and when it lasts longer, it leads to "myocardial necrosis and scarring" with or without the manifestations of "myocardial infarction".

With severe myocardial ischemia and inadequate oxygen supply, fatty acids are not metabolized, and increased anaerobic glycolysis produces lactic acid from glucose, leading to reduced intracellular pH and decreased myocardial stores of ATP and creatine phosphate. Ischemic damage of the myocardial cell membrane causes, increased entry of sodium in to the myocytes and leakage of potassium, also an increase in cytosolic calcium concentration. Myocardial cell damage is reversible if the total coronary occlusion without collateral development lasts $\leq$ 20 minutes, and the damage becomes irreversible with further myocardial necrosis, if the occlusion lasts > 20 minutes.

"Myocardial ischemia" also produces characteristic ECG findings such as repolarization abnormalities, which include "T wave inversion" and, in more severe

cases, "displacement of ST segments". Nontransmural and intramyocardial ischemia, Subendocardial ischemia, and severe transmural ischemia often produce "transient T-wave inversion", "transient ST-segment depression" and "ST-segment elevation" respectively.[3]

Ischemic heart disease can be largely divided in to two categories. Chronic coronary artery disease, which most commonly presents as stable angina pectoris and



acute coronary syndromes (ACSs). ACS further includes acute myocardial infarction with ST segment elevation (STEMI) on presenting electrocardiogram and non-ST segment elevation acute coronary syndrome (NSTE-ACS). The latter includes non-ST segment elevation myocardial infarction (NSTEMI) and unstable angina (UA).[3]

3.5.5 Stable angina pectoris

The stable angina pectoris is an "episodic clinical syndrome due to transient myocardial ischemia". Typically angina is seen in males aged > 50 years, and in females aged > 60 years, with following complaints.

- Episodes of central / substernal chest discomfort: Character of the discomfort is of "squeezing, pressure, heaviness, choking or smothering" type. Rarely, it presents as frank pain.
- Anginal pain usually lasts for 2 to 5 minutes and it is typically "crescendo-decrescendo" in nature.
- Usually radiates to either "shoulder and / or to ulnar border of the forearm and hand". Sometimes can radiate to the "back, interscapular region, root of the neck, jaw, teeth, and epigastrium".
- Precipitating factors: Heavy physical activity, climbing stairs/uphill, emotional stress, sudden exposure to cold, after heavy meal (post prandial angina), bad dreams (nocturnal angina), lying in supine position (decubitus angina).
- Relieving factors: "Slowing or ceasing the physical activities" can relieve the pain in 1 – 5 minutes or "complete rest and/or sublingual nitroglycerin" can relieve even more rapidly.[3]

Physical Examination:

- During the periods when angina patients are asymptomatic, the 'physical examination is usually normal'.

- Evidences of atherosclerosis can be seen elsewhere in the body, such as carotid arterial bruits, diminished arterial pulses in the lower limbs, and aneurysm of abdominal aorta.
- Xanthomas and xanthelasmas as an evidence of risk for atherosclerosis.
- Examination of fundi may reveal an evidence of hypertension such as increased light reflex and arteriovenous nicking.
- Signs of thyroid disorder and / or anemia
- "Cardiomegaly" and abnormal contraction of the cardiac impulse due to "left ventricular dyskinesia" may be found on palpation.
- Auscultation findings: S3 and S4 heart sounds, arterial bruits, and apical systolic murmur due to mitral regurgitation, caused by an impaired papillary muscle function because of an acute ischemia or previous infarction.
- Certain disorders which can cause angina without the coronary atherosclerosis, such as "aortic stenosis, aortic regurgitation, pulmonary hypertension, and hypertrophic cardiomyopathy" must be excluded.[3]

Laboratory tests

- The urine examination may reveal the presence of "diabetes mellitus and renal disease" (such as microalbuminuria), since these conditions accelerate atherosclerosis.
- Blood investigations should include estimation of glucose, creatinine, HbA1C, lipid profile (total cholesterol, LDL, HDL and triglycerides), hematocrit, and, thyroid hormones.

- A chest x-ray may reveal cardiomegaly, cardiac failure or aneurysm of ventricles as the complications of IHD.[3]

ECG findings

- Usually the patients with typical angina pectoris at rest, show normal ECG, yet there may be signs of an old AMI.
- Although repolarization abnormalities, such as "ST-segment and T-wave changes, as well as left ventricular hypertrophy and disturbances of cardiac rhythm or intraventricular conduction" are suggestive of IHD, they are nonspecific.
- More specific ECG changes for IHD are "ST segment depression or elevation, with or without T-wave inversion" that are seen during the episodes of angina pectoris and disappear afterwards.[3]

Electrocardiographic Stress Testing:

- ECG, blood pressure and general condition are monitored while the patient is using a "standard treadmill or bicycle ergometer protocol".
- This test is used not only for the diagnosis of angina, is also helpful for identification of high-risk individuals and assessing the severity of coronary artery disease.
- "Planar or down-sloping ST segment depression of $\geq$ 1 mm" usually indicates myocardial ischemia; "up-sloping ST depression" is less specific.[3]

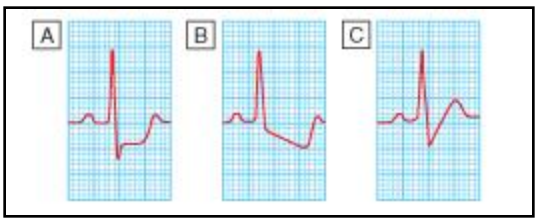


Figure - 7: ECG changes induced by exercise in stable angina pectoris

"A - Planar ST depression is usually indicative of myocardial ischaemia. B - Down-sloping depression also usually indicates myocardial ischaemia. C - Up-sloping depression, however, may be a normal finding".

"Stress echocardiography":

It is an alternate investigation to "myocardial perfusion scanning" and has more or less same predictive accuracy and is more superior to electrocardiographic stress testing. In this technique, ischemic portions of myocardium and infracted areas can be identified using transthoracic echocardiography.[3]

Coronary arteriography:

"Coronary arteriography" provides detailed anatomical information with respect to the "extent and nature of coronary artery disease", in contrast to the functional information provided by stress testing.[3]

3.5.6 Non-ST elevation acute coronary syndrome [Non-ST Elevation Myocardial Infarction (NSTEMI) and Unstable Angina (UA)]

Clinical Presentation:

Typically, chest discomfort is more severe than stable angina and has at least one of the following three features: (1) It occurs at rest or with minimal exertion and lasts >10 minutes; (2) it is of relatively recent onset (i.e., within the prior 2 weeks); and/or (3) it

occurs with a crescendo pattern (i.e., distinctly more severe, prolonged, or frequent than previous episodes). The diagnosis of NSTEMI is established if a patient with these clinical features develops evidence of myocardial necrosis.[3]

History and physical examination:

The chest discomfort is often severe enough to be described as frank pain. chest pain is typically located in the substernal region or sometimes in the epigastrium, and usually radiates to the left arm, left shoulder, and/or neck. The physical examination findings are similar to that of stable angina. If a large portion of myocardium is affected by ischemia or a large NSTEMI, the physical findings can include diaphoresis; pale, cool skin; sinus tachycardia; a third and/or fourth heart sound; basilar rales; and, sometimes, hypotension.[3]

Electrocardiogram:

ST-segment depression can be seen in 20 to 25% of NSTACS patients; it may be transient in patients without biomarker evidence of myocardial necrosis, but may be persistent for several days in NSTEMI. T-wave changes are common but are less specific signs of ischemia, unless they are new and deep T-wave inversions ($\geq$ 0.3 mV).

Cardiac Biomarkers:

Cardiac troponin I or T, which are specific, sensitive, and the "preferred markers of myocardial necrosis", their levels are elevated in NSTEMI patients. The MB isoform of creatine kinase (CK-MB) may also be elevated, which is a less sensitive alternative. Raised plasma levels of these cardiac biomarkers differentiate patients with NSTEMI

from those with UA. Characteristically there is a temporal rise and fall of the plasma concentration of these markers and there exists a direct correlation between the degree of elevation and mortality.[3]

3.5.7 ST – Segment Elevation Myocardial Infarction (STEMI)

Pathophysiology:

STEMI usually occurs when there is an abrupt reduction in coronary blood flow to part of myocardium because of "thrombotic stenosis of one / more coronary vessels previously affected by atherosclerosis". Chronic coronary artery narrowing, especially when it develops gradually do not typically precipitate STEMI because it is frequently associated with development of a rich collateral blood vessel network. Instead, STEMI results when there is a rapid development of thrombus in a coronary artery which is previously affected by atherosclerosis. Cigarette smoking, hypertension, diabetes mellitus and hyperlipidemia are the risk factors for the atherosclerotic vascular injury. In most cases, STEMI occurs when the surface of an atherosclerotic plaque becomes disrupted exposing its contents to the blood and local or systemic conditions favour thrombogenesis. A mural thrombus forms at the site of plaque disruption, and the involved coronary artery becomes occluded. Very rarely, STEMI may also be caused due to the reasons other than atherosclerosis such as, coronary artery spasm (Prinzmetal's angina), coronary thrombi and emboli, coronary ostial stenosis as in aortitis and congenital abonormalities.[3]

Clinical Presentation:

- In up to 50% of STEMI patients, a precipitating factor such as vigorous physical exercise, emotional stress, or a medical or surgical illness will be present.
- Chest pain is the most common symptom. The pain is deep and visceral; Usually patients describe it as heaviness, squeezing, and crushing type.
- Character of the chest discomfort is similar to that of angina pectoris. In contrast to stable angina, it occurs at rest, is more severe, and lasts for much longer period of time.
- The pain is characteristically located in the "substernal region and / or in the epigastrium", often radiating to the left arm, less commonly to the abdomen, back, lower jaw, and neck.
- It is usually associated with sweating, weakness, a sense of impending doom, anxiety, nausea, and vomiting.
- Painless STEMI is more commonly seen in "diabetes mellitus" patients, and it's occurrence increases with age.
- Sometimes "Sudden-onset of breathlessness, progressing to pulmonary edema" may be the presentation of STEMI in elderly patients.
- Profound weakness, confusional state, sudden loss of consciousness and / or arrhythmias, with or without chest pain are the other less common manifestations of STEMI.[3]

Findings on physical examination:

- Majority of STEMI patients are restless and tensed. They frequently try to get rid of the pain by stretching, changing their position and moving about in the bed.
- Sweating, cold extremities and pallor can be the other common observations.
- About 25% of patients with anterior wall MI have features of "sympathetic hyperactivity such as tachycardia and/or hypertension", and around 50% of patients having inferior wall MI present with "parasympathetic hyperactivity like bradycardia and/or hypotension".
- Stroke volume is often decreased, which presents as decreased carotid pulse volume.
- In patients with anterior wall infarction, dyskinetic bulging of infarcted myocardium will produce an "abnormal systolic pulsation in the periapical area", during the first few days.
- Decreased intensity of the first heart sound (S1), paradoxical splitting of the second heart sound (S2), and presence of third (S3) and fourth (S4) heart sounds are the indications of ventricular dysfunction.
- In transmural STEMI patients, a 'pericardial friction rub' can be present. A transient 'midsystolic or late systolic murmur' may be heard in the apical region. [3]

Investigations

Electrocardiogram (ECG):

Usually 'ST segment elevation' is the earliest ECG finding; later on, the size of the 'R' wave decreases, and then 'Q' wave begins to develop if it is transmural (full

thickness) infarction. Subsequently, the inverted T waves appear, which persists even after normalization of the ST segment. Unlike transmural infarction, subendocardial or partial thickness infarction produces ST or T wave changes without prominent ST segment elevation or Q waves.

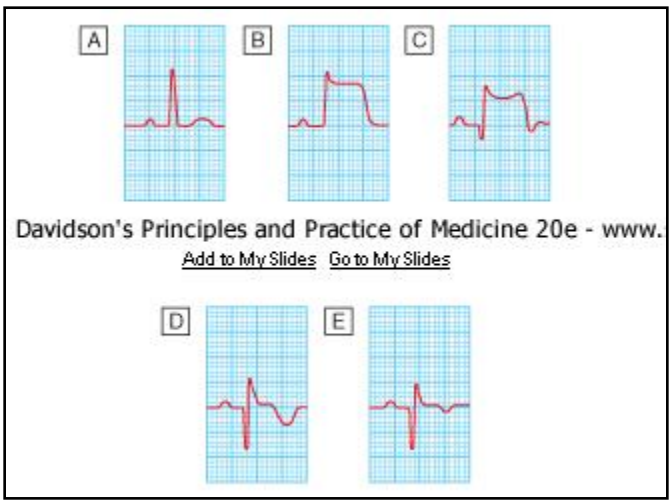


Figure – 8: ECG changes in STEMI "[A] Normal ECG complex; [B] Acute ST elevation; [C] Progressive loss of the R wave, developing Q wave, resolution of the ST elevation and terminal T wave inversion. [D] Deep Q wave and T wave inversion".[14]

The ECG changes in MI can be best seen in the "leads that face the infarcted area". In the case of an 'anteroseptal infarction', ECG changes are seen in "one or more leads from V_1 to V_4". Similarly, 'Inferior wall infarction' produces changes in 'II, III and aVF leads' and 'anterolateral wall infarction' produces changes in "V_4 to V_6, aVL and in lead I". 'posterior wall MI' does not produce ST elevation or Q waves in the standard leads, but produces reciprocal ECG changes i.e. "ST depression and a tall R wave in leads V_1-V_4". [14]

It may be difficult to interpret ECG when history of previous MI and / or 'bundle branch block' is present. In up to one-third of MI cases the initial ECG changes may not be diagnostic.

Cardiac bio-markers:

Certain proteins and enzymes which are normally present in high concentration within the myocardial cells, are released and their level in the blood increases after MI. Currently, cardiac tropoins I & T – the cardiospecific proteins and CK-MB – cardiospecific isoenzyme of creatine kinase, are the most widely employed cardiac markers in the diagnosis of MI. Cardiac troponins T and I are the most sensitive and specific markers of myocardial cell damage currently available. They start to rise after 4 – 6 hours of MI and remain elevated for 10 – 14 days. Plasma CK-MB level starts to increase after 4 – 6 hours, reaches maximum level at around 12 hours and returns to baseline level from 48 to 72 hours after the onset of symptoms.[14]

Other blood tests:

- Increase in the WBC count **(**leucocytosis) is common, and usually found on the 1st day itself.
- Increased "erythrocyte sedimentation rate (ESR)" – Usually remains raised for several days.
- Increase in the plasma concentration of C-reactive protein (CRP)

Chest radiography:

The heart size is usually normal but there may be cardiac enlargement due to pre-existing cardiac damage. Pulmonary oedema may be seen in chest x-ray that is not evident on clinical examination.[14]

Echocardiography:

Echocardiography is very useful in evaluating 'left and right ventricular function'. It can also identify important complications of MI such as mitral regurgitation, cardiac rupture, mural thrombus, ventricular septal defect, and pericardial effusion etc.[14]

3.5.8 Diagnostic criteria of acute myocardial infarction

In the year 1986, World Health Organization established the diagnostic criteria for AMI, in which biomarkers were crucial for the diagnosis. At least two of the following criteria must be met for the diagnosis of AMI: "(1) a history of chest pain, (2) Electrocardiographic (ECG) evolutionary changes, and/or (3) elevations of serum cardiac markers on serial measurements to a level two times the normal value." Over a period of time, it became rare for a diagnosis of AMI to be made without the biochemical evidence of myocardial injury. "A **2000 European Society of Cardiology/American College of Cardiology (ESC/ACC)** consensus conference updated in 2007 (Global Task Force) codified the role of markers by advocating that the diagnosis should be regarded as evidence of myocardial injury based on markers of cardiac damage in the appropriate clinical situation" (Table - 2).[11]

Table – 2: Criteria for the Definition of Acute Myocardial Infarction[11]

1. **Detection of rise and/or fall of cardiac biomarkers (preferably cardiac troponin) above the 99th percentile of the upper reference limit, together with evidence of ischemia with at least one of the following:**
   a. **Ischemic symptoms**
   b. **Electrocardiogram (ECG) changes of new ischemia [new ST-T changes or new left bundle branch block (LBBB)]**
   c. **Development of pathologic Q waves on the ECG**
   d. **Echocardiographic evidence of new loss of viable myocardium or new regional wall motion abnormality**

3.6 Cardiac Biomarkers

We use biomarkers daily in the practice of cardiovascular medicine. Moreover, the use of biomarkers has the potential to continue to improve our ability to provide clinically effective and cost-effective cardiovascular medicine in the years to come. Appropriate risk stratification and targeting of therapies should not only help to improve patient outcomes but also assist in cutting down the unnecessary medical expenditure. In particular, excessive use of imaging biomarkers increases the cost of medical care and can jeopardize patient outcome (for example, radiation exposure or complications of administering contrast material or investigating incidental findings).

Despite of the present usefulness and future promise of biomarkers, a great deal of misunderstanding surrounds their current clinical application.[15]

3.6.1 What is a biomarker?

In the year 1998, National Institutes of Health (NIH) defined biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal

biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."[15]

3.6.2 Medical applications of cardiac biomarkers

1. **Diagnosis:** The use of biomarkers for cardiovascular diagnosis has daily familiarity to practitioners of cardiovascular medicine. The current universal definition of myocardial infarction, for example, requires elevation of a biomarker of myocyte injury, such as cardiac-specific isoforms of troponin.
2. **Risk stratification:** Familiar examples include systolic blood pressure or low-density lipoprotein (LDL) cholesterol. These biomarkers can reliably predict future risk for cardiovascular disorders.
3. **Goals for therapy:** Our contemporary guidelines often specify cut points for targets of treatment—for example, a specific level of a biomarker such as systolic blood pressure or LDL cholesterol in a particular group of individuals.
4. **Targeting of therapy:** Examples of biomarkers used to target therapy include troponin measurements to diagnose acute coronary syndromes patients for early invasive management.
5. **Drug development, evaluation, and registration:** Biomarkers can provide early signals of efficacy that will help prioritize agents more likely to provide benefit on clinical endpoints in large-scale trials.

Biomarkers require rigorous validation before adoption into clinical practice. Clinical use of cardiovascular biomarkers requires a clear understanding of how they should be used.[15]

3.6.3 Characteristics of an ideal cardiac marker

Table - 3: Characteristics of an ideal cardiac marker[16]

High sensitivity	Abundant in cardiac tissue
High specificity	Absent from non-myocardial tissue
	Not detectable in blood from non-diseased subjects
Release	Rapid release for early diagnosis
	Long half-life in blood for late diagnosis
Analytical	Cost effective
	Short turnaround time
	Precise
	Accurate
Clinical	Ability to influence therapy and so improve patient outcome
	Validated by clinical studies

It is important to note that, yet there is no single cardiac marker satisfying all the characteristics of an ideal biomarker.

3.6.4 Clinical Measures of Biomarker Performance

Biomarker evaluation typically involves repeated testing in different settings that include varied patient populations and that use different epidemiologic designs. After discovery by the technologies or identification by a candidate approach, a novel biomarker typically requires development in a translational laboratory for refinement of its assay to address issues of interassay and intra-assay variation before any clinical testing begins.

Beyond simple reproducibility, biomarkers under development for diagnostic, screening, or predictive purposes require further evaluation with a standard set of performance measures that include “sensitivity, specificity, positive and negative

predictive value, discrimination, calibration, reclassification, and tests for external validity". These terms and their use in clinical biomarker development are outlined below.

Sensitivity, Specificity, and Positive and Negative Predictive Value

The validity of a screening or diagnostic test is initially measured by its ability to correctly categorize individuals who have preclinical disease as "test positive" and those without preclinical disease as "test negative." A simple two-by-two table is commonly used to summarize the results of a screening test by dividing those screened into four distinct groups (Fig – 9).

Table – 4: 2 X 2 table to summarize sensitivity, specificity, and positive and negative predictive values

	Disease Present	**Disease Absent**	**Total**
Test Positive	a	b	[a + b]
Test Negative	c	d	[c + d]
Total	[a + c]	[b + d]	
"Sensitivity = a/(a + c)"			
"Specificity = d/(b + d)"			
"Positive predictive value = a (a + b)"			
"Negative predictive value = d/(c + d)"			

a = number of individuals for whom the screening test is positive and the individual actually has the disease (true positives); b = number of individuals for whom the test is positive but the individual does not have the disease (false positives); c = number of individuals for whom the test is negative but the individual actually has the disease (false

negatives); d = number of individuals for whom the test is negative and the individual does not have the disease (true negatives).

In this context, ***sensitivity*** and ***specificity*** provide fundamental measures of the test's clinical validity. Sensitivity is the "probability of testing positive when the disease is truly present" and is defined mathematically as a/(a + c); as sensitivity increases, the number of individuals with disease who are missed by the test decreases, so a test with perfect sensitivity will detect all individuals with disease correctly. In practice, tests with ever-higher sensitivity tend to also classify as "diseased" many individuals who are not actually affected (false positives). Thus the specificity of a test is "the probability of screening negative if the disease is truly absent" and is defined mathematically as d/(b + d). A test with high specificity will rarely be positive when disease is absent and will therefore lead to a lower proportion of individuals without disease being incorrectly classified as test positive (false positives).

A perfect test has both very high sensitivity and specificity and thus low false-positive and false-negative classifications. Such test characteristics are rare, however, because there is a trade-off between sensitivity and specificity for almost every screening biomarker, diagnostic, or predictive test in common clinical use.

In addition to sensitivity and specificity, the performance or yield of a screening, diagnostic, or predictive test also varies depending on the characteristics of the population being evaluated. The ***positive predictive value (PPV)*** is "the probability that a person has the disease of interest, given that the individual tests positive", and is mathematically calculated as PPV = a/(a + b). High PPV can be anticipated, if the disease is common in the study population. Conversely, the ***negative predictive value (NPV)*** is

"the probability that an individual is truly disease free, provided that the test has a negative result", and is mathematically calculated as NPV = d/(c + d). High NPV can be anticipated when the disease is rare in the population being tested.

Discrimination, C-Statistics, and the Receiver Operative Characteristic Curve

Discrimination is the ability of a test to separate those with disease or at high risk for disease (cases) from those without disease or at low risk for disease (controls). The most common method used to measure discrimination has been the area under the receiver operating characteristic (ROC) curve, which relates sensitivity (on the y axis) to (1 − specificity) (on the x axis) across a full range of cut-off values for the test or screening algorithm of interest (Fig. 9).

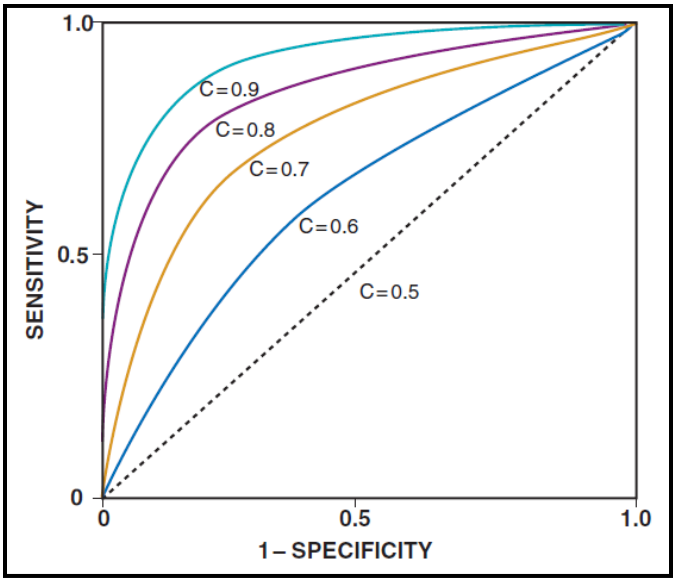


Figure - 9: ROC curves for a series of biomarkers with incremental improvement[15]
The diagonal line corresponds to a random effect (C-statistic = 0.5), whereas the increasing C-statistic corresponds to improving model discrimination.

Given a population of individuals being evaluated, the area under the ROC curve—also called the C-statistic—equals the probability of correctly ranking risk for

individuals by using the test or model under evaluation. A random test with no clinical usefulness would have a C-statistic (or area under the ROC curve) of 0.5, which corresponds to the diagonal line in Figure - 9. A perfect test that completely discriminates individuals with disease from those without disease would have a C-statistic that approaches 1.0. As the C-statistic increases from 0.5 to 1.0, model fit (or test accuracy) improves—thus the change in the C-statistic has been used historically to judge whether a new biomarker can "add" significantly to those already in use. This approach permits direct comparison of the relative efficiency of multimarker panels. This test can aid understanding of the impact that novel pathways and novel risk biomarkers have on prediction and prevention.[15]

3.6.5 Creatine kinase (CK) and CK-MB isoenzyme

Creatine kinase (CK), formerly known as creatine phosphokinase, is an intracellular enzyme, abundant in skeletal muscles, cardiac muscle, and brain; smaller amounts are also seen in other visceral tissues. Damage to the cell membrane because of hypoxia or other cell injury releases CK from the cellular cytosol into the blood. On this basis, elevated serum levels of CK have been used as a sensitive but nonspecific test for myocardial infarction. [17]

Cytoplasmic CK is a dimeric protein, made up of M and/or B subunits. Combinations of these subunits form three isoenzymes namely, "CK-MM, CK-MB and CK-BB". Functions of 'creatine kinase' include, regulation of high-energy phosphate i.e. ATP production and utilization within the contractile tissues; Also helps in shuttling of high-energy phosphate bonds via creatine phosphate from the site of ATP production in

the mitochondria to the site of utilization within the cytoplasm. Hence, the enzyme is found in tissues that have high energy requirements, such as the distal convoluted tubules of the kidney.

CK-MM isoenzyme is most abundant in the skeletal muscle, where it makes around 97% of the total CK and the remaining 2 – 3% is CK-MB. CK-MB is the major isoenzyme in the cardiac muscle, where it forms 15 – 40% of the total CK activity and the remainder is CK-MM. Hence, skeletal muscle damage is associated with increased blood concentrations of CK as well as CK-MB.

The antibodies to the M-subunit of CK have been developed for the specific quantitative assay for CK-MB by immuno-inhibition method. The 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. The residual activity represents CK-MB activity, because CK-BB activity is negligible in serum, unless its concentration is high as in case of severe cerebrovascular accident.

Both serum total CK and CK-MB activities start to rise after 4 – 6 hrs, attain peak levels between 12 – 24 hrs and return to baseline level between 48 – 72 hrs after the myocardial damage. The level of serum CK-MB activity is significantly more cardiac specific when compared to that of total CK.[16]

'CK-MB' was the biomarker of choice for the diagnosis of MI until the advent of cardiac troponin assays. Its major limitation is relative lack of specificity, because it is found in skeletal muscle, tongue, diaphragm, small intestine, uterus, and prostate.[2] Hence,

the CK-MB level may be falsely elevated in several clinical situations. The more common settings include certain neuromuscular disorders (e.g. muscular dystrophy), myocarditis, cardiomyopathy, rhabdomyolysis, prolonged ischaemic skeletal muscle exertion (e.g. in marathon runners or military recruits), and in alcohol abuse or trauma.[18] Use of the CK-MB relative index i.e. the ratio of CK-MB to total CK partially addresses this limitation for skeletal muscle as a source.[2]

Creatine kinase isoforms

Two isoforms of the CK-MB isoenzyme have been identified in the blood. The tissue form of CK-MB is called as CK-MB2. CK-MB1 isoform is produced after the the enzyme carboxypeptidase- N removes of the "lysine residue from the carboxy terminal of the M-subunit". Normally, two CK-MB isoforms exist in equilibrium (1:1 ratio) in the plasma. Following the myocardial tissue damage, CK-MB2 concentration increases in the plasma, altering the 'CK-MB2:CK-MB1 ratio' from 1:1 to 2:1 which is identified by 'high-voltage gel electrophoresis'. Considerably increase in the CK-MB2:CK-MB1 ratio in plasma can be detected between 2 and 4 hrs after myocardial infarction. Systematic prospective research studies have found that, CK-MB isoforms ratio is an early marker of myocardial injury, and CK-MB2:CK-MB1 ratio above 1.5:1 is diagnostic of MI. The isoform ratio returns to baseline level between 18 – 30 hrs after myocardial damage. Unfortunately, high-voltage gel electrophoresis technique used for the separation and measurement of CK-MB isoforms is impractical for routine use because it requires specialist equipment and a technical expertise. In addition, as CK-MB is also found in skeletal muscle in small amounts, any skeletal muscle damage can lead to rise in the CK-MB isoform ratio.[16]

Future of CK-MB

Several groups have recommended for the elimination of CK-MB assays. The reason for such an advocacy comes from the thought that, CK-MB estimation increases unnecessary economic burden on the patient while not contributing to the diagnostic value, also the doctors who depend on CK-MB for chest pain evaluation, often do injustice to the patients. In addition, these assays restrict the doctor's ability to learn effective utilization of cardiac troponin assays, which would be more valuable in almost every situation. Accordingly, laboratories have to consider seriously on discontinuing the use of CKMB.

Those who suggest for continuing the use of CK-MB for AMI diagnosis, emphasize on following situations that are worthy to note. The first includes, the 'diagnosis of recurrent infarction after the primary AMI'. Initially, when the guidelines for the use of cardiac troponin (cTn) were laid down, many raised questions over usefulness of cTn for diagnosing recurrent myocardial infarction because cTn elevations persist for so long (7 – 10 days). Recent studies suggest that cTn values can diagnose acute recurrent infarction as well, because re-infarctions are associated with clinically significant re-elevations in cTn levels that can be detected promptly. Both the tests require serial measurements over frequent time intervals. If this is done, CK-MB would not have any peculiarity that would make it superior to cTn.

Other advantage of CK-MB over cTn which is often highlighted is concerned with diagnosis of MI patients with renal failure. There exists no such advantage because prior data suggest that elevated CK-MB levels have been observed in up to 20% of renal

failure patients, might be because of coexisting skeletal muscle myopathy. Similarly it has been claimed that, CK-MB has edge over cTn for the 'diagnosis of MI in patients after severe exercise', because cTn elevations are known to occur in these individuals. However, this advocacy has not considered the extensive research literature, evidencing significantly increased CK-MB levels in the blood in almost all individuals after severe exercise.[11]

3.6.6 Cardiac Troponins

Troponins are the regulatory proteins of the contractile proteins in myofibrils of skeletal and cardiac muscles (Figure - 10). The troponin complex consists of three protein subunits namely, troponin I (the inhibitory component), and troponin T (the tropomyosin-binding component) and troponin C (the calcium-binding component). Its function is the regulation of striated and cardiac muscle contraction. The complex regulates the calcium-modulated interaction between actin and myosin on the thin filament. The function of cTnI (molecular weight 26 kDa) is to inhibit actinomyosin ATPase activity. TnC (molecular weight 18 kDa) interacts tightly with cTnI, reversing the inhibitory effect. The strength of the interaction depends on the degree of saturation of calcium binding sites on the TnC molecule; each TnC molecule has four calcium binding sites. There are various isoforms of troponin subunits. These isoforms differ in their location. Troponin C has two isoforms, one in skeletal muscle and another in human heart. Since heart and skeletal muscle isoforms of TnC are identical, it is not useful as a cardiac specific biomarker. "Cardiac specific troponin T (cTnT) and cardiac-specific troponin I (cTnI)" have tissue specific isoforms that are encoded by unique genes. Troponins are predominantly (94 to 97%) bound to the myofibrils, with a smaller fraction (3 to 6%) present in cytoplasm.

Cardiac TnI and TnT and their skeletal muscle counterparts are encoded by different genes and hence have distinctive amino acid sequences. Human cTnI is completely cardiac specific because it has an extra 31 amino acid chain on the amino terminal, in contrast to skeletal muscle TnI. Only a single isoform of cTnI has been identified. Also, cTnI has never been isolated from normal, regenerating, or diseased human or animal skeletal muscle. cTnT is also a cardiac specific marker because it has a unique 11 amino acid residue at amino-terminal end and is encoded by a gene, which is different from the gene for skeletal muscle isoforms. However, cTnT is found to be expressed in minor quantities, in skeletal muscle, during human fetal development, in regenerating rat skeletal muscle, and in diseased human skeletal muscle.[11]

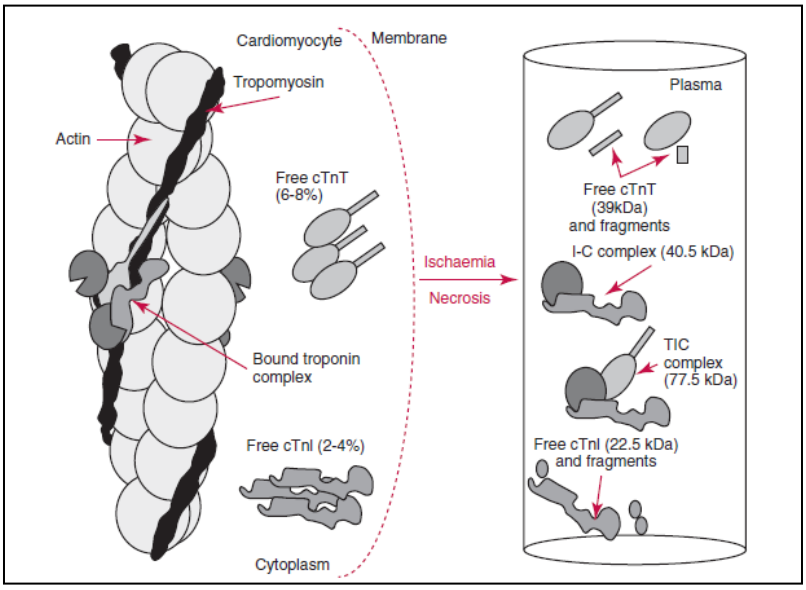


Figure – 10: Structure of cardiac troponin complex; and Different forms of troponins released after myocardial damage[11]

Cytoplasmic fraction of cTn, which accounts for 3% to 5% of cTnI and 7% of cTnT is released first in to the peripheral circulation within 4 – 6 hrs of damage to the

cardiac myocytes; the release of myofibril bound cTn subunits contributes to the continued rise in the blood which remains detectable for days (4–7 days for cTnI and 10–14 days for cTnT). cTns are fragmented into smaller molecules primarily by the reticuloendothelial system, that are cleared by renal excretion. Initial rise in the cardiac troponin level after the ischemic myocardial damage is delayed, because of the fact that, atleast 2 – 4 hours of time is required for the myocardial necrosis to develop. Hence, cardiac troponins can be detected in the blood only after this latent period after the cardiac ischemia. Consequently, serial measurement of cardiac troponins at the time of presentation and again after 6–9 hrs from the onset of ischemic symptoms, has been recommended.[19]

Several earlier research studies have shown the poor sensitivity of conventional cTn assays for the early diagnosis of AMI. In one of such studies, the sensitivities of cTnT and cTnI at the time of presentation was found to be 33.3% and 3.7% respectively. On serial measurement, sensitivities increased to 89% and 82% at 6 hrs and to 96% and 89% at 12 hrs respectively. At presentation, the specificities of cTnT and cTnI were high i.e. 89% and 98% respectively. There was no significant increase in the specificities with serial measurements. There was a significant improvement in the PPV and NPV values of cTnT and cTnI, on serial measurement from the time of presentation to 12 hrs after. Hence, they concluded that, cardiac troponins have maximum utility in detecting AMI no less than six hours after presentation.[20]

Immunoassays

Radio-immuno assay (RIA) to measure cTnI, using polyclonal anti-cTnI antibodies was first developed by Cummins and coworkers. Later on, Bodor and Ladenson described the first monoclonal anti-cTnI antibody–based enzyme-linked immunosorbent assay (ELISA). Several manufacturers have now developed diagnostic immunoassays based on monoclonal antibody for the measurement of cTnI in the serum. In clinical practice or research, switching over from one assay to another is difficult because of two hurdles. First, no primary reference cTnI material is yet available for manufacturers for the standardization of cTnI assays. Second, because cTnI is seen in its various forms in the blood and the different antibodies used in the currently available assays bind to different epitopes of cTnI, the assay concentrations are not consistent.

Fractionation of cTnT from the plasma of AMI patients demonstrated that, initially, 'free cTnT' was released, followed by the release of 'ternary troponin T/I/C complex', with a small amount of 'free TnT'. Fractionation of cTnI from the plasma of AMI patients demonstrated that the 'cTnI/TnC complex' is the predominant form that was released. The majority of 'free cTnI' was released initially within short time after the injury. A small concentration of 'cTnI/ cTnT complex' was also found.

The presence of "multiple forms of cTnI" in plasma influences the development of assay procedures, and their analytical performance. All commercial assays available for cTnI measurement are immunoassays, hence selection of antibody is important. Various forms of cTnI present in the plasma (free cTnI, T/I/C, I/C and I/T) may be oxidized, reduced or phosphorylated to varying degrees, and/or proteolytically degraded.

Antibodies selected for immunoassays must, not undergo any modifications in vivo, identify epitopes on the cTnI molecules and able to bind to all circulatory forms of troponin complexes equally. Also, the use of proper calibrator / standard is equally important. Because a definitive cTnI standard material is not yet developed, immunoassays of different manufacturers cannot be compared, and the cTn concentration values obtained differ by up to 20 fold. The sensible utilization of patent law has guaranteed that one single manufacturer delivers all cTnT kits, so that, between technique calibration issues don't happen.[11]

Defining normal reference limits for cardiac troponins

Consensus guidelines from the "Global Task Force for the Universal Definition of Myocardial Infarction and the National Academy of Clinical Biochemistry (NACB) and the updated American College of Cardiology / American Heart Association and Epidemiology" guidelines have recommended that, "at least 1 cTn concentration in the blood more than 99th percentile value in patients during the first 24 hours after onset of ischemic symptoms indicates myocardial necrosis". It is recommended that cTn assays should be carried out with appropriate quality control procedures and 99th percentile limit of coefficient of variation (CV) $\leq$ 10%. [11]

Causes of acute cTn elevation other than acute ischemic heart disease

It is pertinent to know that, an elevation in the serum cardiac troponins indicates the damage to myocardial cells, but not it's mechanism. Myocardial cell injury can occur in cardiac conditions other than ischemic heart disease, such as

1. Trauma: Contusion, slow potential cardiac ablation, pacing, ICD firings, cardioversion, myocardial biopsy, and various cardiac interventions.
2. Congestive heart failure
3. Severe "valvular heart diseases", with volume or pressure overload.
4. Hypertension: Left ventricular hypertrophy (LVH) or cardiomegaly associated with hypertension can increase cardiac wall stress and reduce nutritive perfusion, and causes increases in cTn.
5. cTn level is also found to be elevated in noncardiac surgery patients during the post-operative period, commonly in 'vascular surgery patients' who have been best studied, which may be because of the underlying coronary heart disease associated with hypertension or hypotension and/or tachycardia, anemia, or other similar causes with defective myocardial oxygen supply.
6. Elevated cTn level is often seen among renal failure patients
7. Critically ill patients with or without underlying coronary heart disease
8. Carbon monoxide poisoning.
9. Snake bite venom can be another cause.
10. Inflammatory heart diseases: Myocarditis, pericarditis
11. Pulmonary embolism
12. Severe septicemia with hypotension
13. Severe burns
14. Systemic rhabdomyolysis, dermatomysitis and polymyositis
15. Severe exercise
16. Cardiotoxic drugs (chemotherapy and alcohol)[11]

There has been a misunderstanding that, elevation of cTn is only because of the myocardial injury and necrosis. Atleast six mechanisms have been proposed in order to explain the release of cardiac troponins into the blood circulation which include, myocardial cell necrosis, apoptosis, normal myocytes turnover, proteolytic degradation, increased cell membrane permeability and formation of membranous blebs. In addition, there may be other potential, yet not explained mechanisms involved in the release of cTn. For example, it is still unknown why cTn is elevated in certain extra-cardiac diseases such as sepsis. Whether cTn is elevated in ischemia in the absence of myocyte necrosis remains controversial.

There is a risk of misinterpretation of elevated troponin results. Almost 13% of patients presenting with chest pain and elevated high sensitive cardiac troponin (hs-cTn) and eventually prove not to have ACS. Hs-cTn can be elevated in patients with various cardiac and non-coronary cardiovascular co-morbidities.[21]

3.6.7 Myoglobin

'Myoglobin' is relatively small, 'haem protein' present in both cardiac and skeletal muscle cytosol, comprising around 2% of the total cytosolic muscle protein. After the damage to the myocytes, it is rapidly released into the blood because of its presence in the cytosol and relative low molecular weight (17 kDa). Plasma concentration of myoglobin increases within 2 – 3 hrs after myocardial injury, usually peaks within 6 to 9 hrs, and comes down to normal within 24 hrs. The association between myoglobinaemia and AMI was first reported in 1975. Myoglobin has low cardiac specificity because the myoglobin present in both skeletal and cardiac muscle has

identical amino acid sequence. Hence, myoglobin is also elevated in skeletal muscle damage such as skeletal muscle or neuromuscular disorders, strenuous exercise, intramuscular injections. Decreased renal clearance because of renal insufficiency also raises myoglobin levels in the blood.

Until recently, radioimmunoassay was the only available assay for myoglobin measurement, which is time consuming and have health hazards. However, a number of rapid immunoassays for myoglobin have been developed subsequently which have helped the myoglobin for becoming an 'early marker of myocardial damage'. "The National Academy of Clinical Biochemistry in the US, and the Joint European Society of Cardiology (ESC)/American College of Cardiology (ACC) Committee for the Redefinition of Myocardial Infarction", have advocated to utilize the plasma myoglobin and / or CK-MB isoforms diagnosis of AMI during the early hours.[16]

A single myoglobin assay at presentation for diagnosing AMI among patients with suspected ACS has been shown to have a sensitivity of 70% and a NPV of 97.4%.[22] In another large study involving emergency department patients with suspected ACS found that, the initial sensitivities of myoglobin and cTnI at presentation were 60% and 52% respectively but the sensitivity increased to 94%, when serial measurement of combination of cTnI and myoglobin are done over a period of 9 hrs.[23]

Although myoglobin is the earliest indicator of myocardial cell damage, it is not the perfect cardiac marker for diagnosing AMI because of lack of specificity. Myoglobin may be most helpful when used in conjunction with other specific cardiac markers such

as cTns in the rapid diagnosis of AMI, especially in patients with atypical chest pain or nonspecific ECG changes.[24]

3.6.8 Heart-type fatty-acid binding protein (H-FABP)

The 'fatty acid binding proteins' (FABPs) are a family of structurally similar, cytosolic proteins. 'Ockner' in the year 1972 discovered FABPs, during his studies on absorption of fatty acids in the intestine. They have high affinity for the non covalent binding with fatty acids - hence called FABPs. These proteins are widely distributed in the body. They are particularly abundant in actively fatty acid metabolizing tissues such as, heart and liver. Several other species, including insects, fish, and birds are also reported to have FABPs. There are nine types of FABPs identified. All FABPs have low molecular weight (12–15 kDa), but they are different with respect to their "tissue distribution, concentration within tissue, isoelectric point (PI), binding capacity, and binding specificity".[7]

FABPs are cytoplasmic proteins, made up of 126–134 amino acids, having a molecular weight of 14 to 15 kDa. There are at least nine different FABP types, which are named after their first tissue of isolation (Table – 5). Some of the tissues contain several types of FABPs. 'Heart type fatty acid binding protein (H-FABP)' is most widely distributed in the body. It is mainly located in 'heart', with minor concentrations in 'skeletal and smooth muscle cells, mammary epithelial cells, aorta, distal tubules of the kidney, lung, brain, placenta and ovary' (table – 6). Different types of FABPs have 22 – 73 percent similarity in the amino acid sequence, but they have highly conserved three dimensional structures.[25]

Table – 5: Tissue distribution of FABP types[25]

FABP type	Abbreviation	Tissue
Liver	L	Liver, kidney, intestine, stomach
Intestinal	I	Intestine and stomach
Heart	H	Heart, skeletal muscle, kidney, aorta, brain, adrenals, testes, placenta, ovary, mammary gland, stomach, lung
Adipocyte	A	Adipose tissue.
Epidermal	E	Skin, brain, capillary endothelium, lens, retina
Ileal	IL	Intestine, stomach, adrenals, ovary
Brain	B	Brain
Myelin	M	Peripheral nervous system
Testicular	T	Testis

Table - 6: Overview of reported tissue contents of different human FABP types in the heart, skeletal muscle, intestine, liver and brain[26]

Tissue	Part	H-FABP (µg/g ww)	L-FABP (µg/g ww)	I-FABP (µg/g ww)	B-FABP (µg/g ww)
Heart	Epicardial	540	--	--	--
	Midcardial	600	--	--	--
	Endocardial	550	--	--	--
Skeletal muscle		173	--	--	--
Liver		--	2700	--	--
Small intestine	Duodenum	3.5	124	2.22	--
	Jejunum	4.9	198	4.79	--
	Ileum	3.2	58	1.04	--

	Colon	2.7	26	0.27	--
Brain	Frontal lobe	26.3	--	--	3.1
	Temporal lobe	31.9	--	--	2.2
	Occipital lobe	21.2	--	--	1.0
	Cerebellum	16.2	--	--	2.8

Heart type fatty acid binding protein (H-FABP)

H-FABP is a small, cytoplasmic protein with a molecular weight of 15 kDa. It consists of 132 amino acids. It is one of the most abundant proteins in the heart, comprising 5–15% of the total cytosolic protein pool, equivalent to 0.5 mg per gm wet weight of cardiac tissue. The gene encoding H-FABP (FABP 3 gene) is present on chromosome 1 with its specific location being 1p33-p32.[7]

Structure of H-FABP

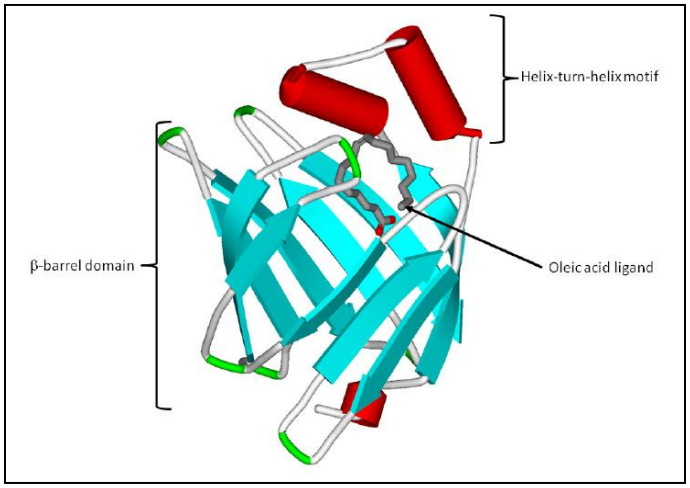


Figure – 11: The crystal structure of human heart FABP (HFABP) containing an oleic acid ligand[27]

All FABPs have in common a 10-stranded anti-parallel β – barrel structure which is formed by two orthogonal five-stranded β – sheets (Figure – 11). The ligand binding

pocket is located inside the β – barrel, and is framed on one side by the N-terminal helix-turn helix motif, consisting of two short α – helices between the first two β – strands, that is thought to act as the major portal for long chain fatty acid (LCFA) entry and exit.[27]

Functions of H-FABP

Heart requires fatty acids, as the major source of energy. Normally, fatty acid oxidation provides 50–80% of the heart's energy requirement and contributes for 10% of the total body turnover of fatty acids. But, very less amount of fatty acids (only 0.1% of total body fatty acid synthesis) are synthesized in the heart.

1. Cytoplasmic H-FABP acts as the equivalent of plasma albumin, required for the 'intracellular transport of insoluble fatty acids'. H-FABP binds with two fatty acids molecules. It is required for the transport of 'fatty acyl coenzyme A' in to the mitochondria for oxidation with the generation of energy.
2. H-FABP mediates fatty acid signal translocation to peroxisome proliferator activated receptors (PPARs) thus helps in regulation of gene expression.
3. Increased fatty acid concentration inside the myocardial cells as a result of myocardial ischemia, can be harmful to the heart because of the detergent like effects of locally high concentrations of long chain fatty acids. The presence of H-FABP gives protection to myocardial cells, against the detergent like action of fatty acids, without affecting the availability of these fatty acids for the cellular metabolic requirements.
4. Some studies have reported that, H-FABP can act as free radical scavenger during the period of myocardial ischaemia.[7]

In view of the importance of H-FABP in myocardial function, it is quite but natural that its measurement in the plasma could be a reliable test for myocardial ischemia.

Only the cardiac muscle contains the high concentration of H-FABP. But, because H-FABP is present in very low concentration in other tissues of the body as well, it is not entirely cardiac specific. The concentration of H-FABP in skeletal muscle varies from 0.05 to 0.2 mg/g wet weight of muscle tissue. It is also present in other tissues such as, brain, kidney, placenta, adipose tissue, adrenal glands, stomach, mammary glands, testes, and aorta, in very low concentrations. But, the identification of H-FABP in these tissues does not really imply that, it is available in all the cells of that tissue.

The FABPs isolated from heart, liver and intestine share 20 – 35% similarity in the sequence of amino acids. FABPs from heart, adipose tissue and nerve share 60 – 80% similarity in the sequence of amino acids. Earlier studies have shown that, antibodies produced against heart, liver, or intestinal FABPs may thus have ‘cross reactivity of up to 5%’ with each other, and around ‘1 ng/ml detection limit’. Cross reactivity with other FABP can be another possible reason for the detection of H-FABP in various other tissues. H-FABP assays with improved sensitivity, with lower detection limit as low as 0.25 ng/ml have been developed recently. The cross reactivity of these assays with other tissue FABPs is also very low (< 0.005%). These newer assays can be used to know the true tissue distribution of H-FABP.[7]

The logical basis for H-FABP, as an early and accurate marker of myocardial injury

Following are the logical reasons behind the use of H-FABP as an early and accurate marker of myocardial injury. (i) 'High concentration of H-FABP' in myocardial tissue; (ii) H-FABP is virtually confined to the cytoplasm; (iii) H-FBAP has relatively small molecular weight (15 kDa); (iv) Relative cardio-specificity of H-FABP – its distribution pattern in tissues other than heart more or less similar to that of CK-MB, and (v) Earlier release of H-FABP into the blood (< 2 hrs), after the onset of myocardial injury.[7]

H-FABP assays and normal range

Initial method for the measurement of H-FABP was based on "sandwich enzyme-linked immunosorbent assay (ELISA) using two monoclonal antibodies specific for H-FABP". Later on various other sensitive methods were developed which include, enzyme immunoassay (EIA), microparticle enhanced immunoassay, fully automated latex-agglutination assay, qualitative lateral-flow assay, and Immuno-turbidimetric assay.[28] The reported normal reference ranges of H-FABP in serum and plasma are 'assay and method dependent'. In the year 1991, Tanaka et al. found that the normal level of H-FABP as less than 2.8 mg/l. Later on, Tsuji et al. and Wodzig et al. reported the normal limit of H-FABP as 0.0–0.6 mg/l and 0.3–5 mg/l in the year 1997 and 1993, respectively. Under normal conditions, H-FABP is present in a very low concentration in the blood circulation, which may be because of the damaged skeletal muscle cells release this protein.[7]

Plasma H-FABP and diagnosis of acute myocardial infarction:

In the year 1988, H-FABP was introduced by Glatz JFC as a new promising cardiac bio-marker for the diagnosis of AMI during the early phase. Normally, H-FABP is not present in the blood or interstitial fluid, but after myocardial cell injury it is released into the blood. The normal concentration of H-FABP in the plasma is less than 5 mg/l. The "cytoplasmic to vascular concentration ratio" of H-FABP is about 200 000:1. This makes H-FABP a better marker for the 'early diagnosis and quantification of myocardial injury'. The H-FABP starts to rise in the blood in less than 2 hrs after the myocardial damage, level peaks at around 4 – 6 hrs, and returns to normal level within 20 hrs. H-FABP is shown to have sensitivity of >80% for the diagnosis of AMI within 30–210 min after the onset of symptoms. Between 0–6 hrs after the onset of ischemic symptoms, the conventional cardiac markers such as cardiac troponins (cTnI & cTnT) and CK-MB mass or activity, and will only be starting to rise in the blood, and their sensitivity has been reported to be around 64%.[7]

Limitations:

FABPs in the human heart and skeletal muscle are shown to be similar to one another. Hence, during the course of AMI any injury to the skeletal muscle, as in cases of traumatic cardiopulmonary resuscitation, electric cardioversion and intramuscular injections, etc., results in the release of skeletal muscle FABP, and this can affect the H-FABP assay reports. Increased plasma concentration of H-FABP has also been reported in cardiac as well as non-cardiac surgeries. Since, H-FABP is cleared through kidneys, the plasma H-FABP level may be elevated in renal disorders. However, renal failure can

be readily detected by routine biochemical tests and it should not be a major confounding factor for the diagnosis of MI with H-FABP.[7]

3.7 Review of earlier related research studies

Several studies have been conducted in the past two decades with regard to the usefulness of "heart type fatty acid binding protein" in the diagnosis, quantification of the infarct size, and in the assessment prognosis of myocardial damage in ischemic heart disease patients and arrived at conflicting findings. There has been a considerable clinical interest in whether the measurement of H-FABP would be useful as an index of myocardial infarction, particularly within 6 hours after the onset of symptoms.

Takao Tanaka et al. in 1991 studied the appearance and time-course changes of H-FABP levels in the serum and urine samples of AMI patients after developing a competitive enzyme immunoassay (C-EIA) for the estimation of serum and urinary H-FABP concentration. Serum and urinary H-FABP levels in 86 controls without AMI, were found to range between 0 and 2.8 ng/mL. Serial measurements of H-FABP were performed on 11 patients with AMI in serum and urine samples. They found that, serum and urinary H-FABP levels were significantly higher in the first samples collected within 14 hrs after the onset of symptoms. H-FABP levels were also higher in 2 serum and 2 urine samples collected only 1.5 hrs after the onset of symptoms, while in the same serum samples the CK-MB activity was still normal. Peak H-FABP levels in the serum and urine were observed between 5 and 10 hrs after the onset of symptoms, and thereafter returned back to normal levels. Peak levels of H-FABP in both serum and urine observed earlier than that of CK-MB activity in serum. Hence they concluded that, the presence of

H-FABP in serum and/or urine seems to be a marker for myocardial damage and could be used as an useful tool for the early diagnosis of AMI.[29]

In the year 1992, Kleine AH et al. evaluated the release of cytoplasmic H-FABP into the plasma up to 38 hrs after the onset of the first clinical symptoms of AMI patients using a sensitive direct and noncompetitive Enzyme Linked Immunosorbent Assay of the antigen capture type (sandwich ELISA). Plasma H-FABP levels were compared with plasma CK-MB and alpha-hydroxybutyrate dehydrogenase (α-HBDH) activities. H-FABP levels were significantly elevated above their threshold level within 3 hrs after the onset of symptoms of AMI. Peak levels of H-FABP, CK-MB and α-HBDH were reached at 4.1 ± 0.9 hrs, 8.4 ± 1.4 hrs and 25.0 ± 9.5 hrs (mean ± SD, n= 10) after AMI, respectively. Serial time curves of the plasma contents of H-FABP reveal that after myocardial infarction, H-FABP is released in substantial amounts from human hearts. Plasma H-FABP, CK-MB and α-HBDH levels were at or above the threshold level in blood-samples taken within 3.5 hrs after the first onset of symptoms of AMI, in 18, 9 and 6 patients out of 22 with established AMI respectively. These findings suggest that, for the early detection of acute myocardial infarction in man cytoplasmic H-FABP is more suitable than CK-MB and/or alpha-hydroxybutyrate dehydrogenase.[30]

In the year 1993, Ryoichi Tsuji et al. assessed the usefulness of estimation of serum and urinary concentration of H-FABP as an early marker of AMI, in 63 AMI, 24 unstable angina and 10 chest pain syndrome patients, within 6 hrs after the onset of symptoms. 91.4% of AMI patients tested within 3 hrs and 100% (111 / 111) of AMI patients tested between 3–6 hrs of onset of symptoms showed increased serum H-FABP

levels. 88.9 % of AMI samples within 3 hrs and 75 % at 3–6 hrs of AMI showed elevated H-FABP levels in the urine. 20 % (8 / 40) and 66.3 % (53 / 80) of serum samples at 0–3 hrs and 3–6 hrs, showed higher CK-MB activity respectively. Serum samples which were positive for CK-MB, always showed high H-FABP level and also H-FABP elevation occurred much earlier to CK-MB. 17.8 % (5 / 28) of chest pain syndrome patients and 56.7 % (34 / 60) of unstable angina patients showed increased H-FABP levels at 0-6 hrs, but the H-FABP levels were lower than AMI. Thus, 'H-FABP may be a valuable indicator for the diagnosis of hyperacute myocardial infarction'.[31]

In the year 1994, Jan F C Glatz et al. did a study on 49 patients with AMI who had been treated with thrombolytic agents within six hours after the onset of AMI. H-FABP was measured immunochemically and CK-MB and α-hydroxybutyrate dehydrogenase (HBDH) levels were enzymatically assayed in serially collected plasma samples. Cumulative protein release into plasma was calculated using previously validated circulatory models and a value of 2.6 h^{-1} for the fractional clearance rate of H-FABP from plasma. They found that, release of H-FABP was completed earlier (24-36 hrs) after AMI in comparison to that of CK-MB (50-70 hrs) and HBDH (> 70 hrs). However, infarct size estimated from the cumulative release of the proteins, yielded a comparable value of 4-6 gram equivalents of healthy myocardium per litre of plasma for both H-FABP and the two enzymes. From these findings they concluded that, H-FABP released from the damaged myocardial cells after AMI is quantitatively recovered in plasma and that H-FABP is a useful biochemical plasma marker for the estimation of myocardial infarct size in humans.[32]

In the year 1995, Kenro Yoshimoto et al. measured the content of H-FABP in various tissues in human beings, by "sandwich ELISA". In the cardiac muscle, ventricles had higher content of H-FABP (0.46 mg / gm of wet weight) than the atria (0.25 mg / gm of wet weight). H-FABP constituted 1.5% of the total cytosolic protein in the ventricles, and 0.7% of the total protein in the cytosol of atria. Among the skeletal muscles, around $1/4^{th}$ H-FABP content of the cardiac muscle was found in diaphragm, where as very low concentration of H-FABP was detected in other skeletal muscles. Among the other organs of the body, kidneys contained < $1/10^{th}$ of H-FABP content of the heart and small intestine and liver had only negligible amounts. The tissue distribution pattern of H-FABP in cardiac and skeletal muscles was inversely related to that of myoglobin, and was similar to that of CK-MB activity. In both AMI cases and controls, the plasma "myoglobin/H-FABP ratio" closely reflected that in the cardiac and skeletal muscles. They concluded that, "H-FABP may be useful as a specific marker of AMI, and the plasma myoglobin/H-FABP ratio could provide additional valuable information for the diagnosis of AMI".[33]

In the year 1997, Junnichi Ishii et al. compared the diagnostic utility of serum H-FABP and myoglobin concentrations, and their ratio for the early diagnosis of AMI. They included 165 AMI patients within 6 hrs of onset of chest pain and in 104 healthy controls in the study. Serum H-FABP had greater sensitivity [81.8%, 95% CI 574.2 – 89.4%], specificity [86.4%, 95% CI 5 78.1 –94.6%], and predictive accuracy [83.6%, 95% CI 5 78.0 – 89.3%] for the diagnosis of AMI than that of myoglobin. H-FABP [0.946, 95% confidence interval (CI) 50.913– 0.979] and myoglobin [0.895, 95% CI 50.846–0.944] had significantly greater area under ROC curve, when compared to that of ratio between

myoglobin and H-FABP [0.823, 95% CI 50.765 – 0.881]. They concluded that, "H-FABP is a more sensitive and specific marker than myoglobin for the early diagnosis of AMI within 6 hrs of onset of symptoms, and that their ratio cannot give an added advantage over the measurement of H-FABP alone".[34]

In the year 1998, Jan F.C. Glatz et al. analyzed blood samples collected immediately upon admission to the hospital (<6 hrs after AMI) from 83 patients with confirmed AMI. H-FABP had significantly greater diagnostic sensitivity (78%, confidence interval 67–87%) than for myoglobin (53%, CI 40–64%) ($P<0.05$). In addition, the differences in contents of myoglobin and H-FABP in heart and skeletal muscles and their simultaneous release after muscle injury allow the plasma ratio of myoglobin/H-FABP to be applied for discrimination of myocardial (ratio 4–5) from skeletal muscle injury (ratio 20–70). They concluded that, rapid and more sensitive immunochemical assays for plasma H-FABP are now being developed and soon this will enable the introduction of this marker in routine clinical practice.[35]

In the year 2000, Fumio Okamoto et al. assessed the diagnostic utility of H-FABP for the diagnosis of AMI in the early phase. 140 AMI patients, 49 non-AMI chest pain patients and 75 healthy controls were included in the study. Blood samples collected within 12 hours after the onset of symptoms were analyzed for serum levels of H-FABP and compared with myoglobin and CK-MB. H-FABP [92.9%] had greater sensitivity, when compared to that of myoglobin [88.6%] and CK-MB [18.6%] within 12 hrs. The diagnostic efficacy rates of H-FBAP, myoglobin and CK-MB were 86.2%, 80.4% and 39.6% respectively. Also, area under the ROC curve [diagnostic validity] for H-FABP

[0.921] was significantly higher than for myoglobin [0.843] and CK-MB [0.654]. Also, the "sensitivity, specificity, diagnostic efficacy and diagnostic accuracy" of above tests, in patients with chest pain within 3 hours and /or 6 hours after the onset of symptoms, were almost similar to those patients within 12 hours after symptoms. Thus they concluded that, "H-FABP was more sensitive than both myoglobin and CK-MB, more specific than myoglobin for detecting AMI within 12 hours after the onset of symptoms, and shows the highest values for both diagnostic efficacy and ROC curve analysis. H-FABP has a great potential as an excellent biochemical cardiac marker for the diagnosis of AMI in the early phase".[36]

T. Petzold et al. in the year 2001, studied the value of H-FABP, in assessing the peri-operative myocardial injury. 32 consecutive patients undergoing coronary artery bypass grafting were included in a prospective, randomized study using standard operative procedures (SOP) and myocardial protection. Three patients with perioperative myocardial infarction were also included in the study. Serial blood samples collected preoperatively, before ischemia, 5 and 60 min after declamping, 1 and 6 hrs postoperatively and on postoperative days 1, 2 and 10 and were tested for H-FABP, CKMB and TnI. They found that, there was no hospital mortality among the study participants. In routine patients, H-FABP levels peaked as early as 1 hr after declamping, whereas peak levels for CKMB and TnI were observed only 1 hr after arrival in the intensive care unit. Patients with perioperative infarction showed peak levels of all marker proteins few hours later. There was a significant correlation between peak serum levels of H-FABP with peak levels of CKMB (r=0:436, P=0:011) and TnI (r=0:548, P=0:001), indicating the degree of myocardial damage. They concluded that, H-FABP is

a rapid marker of perioperative myocardial damage and peaks earlier than CKMB or TnI. The serial measurement of marker proteins immediately after reperfusion is more suitable for the detection of perioperative myocardial infarction than a fixed cut-off level in single sample.[37]

In the year 2003, Nakata T et al. did a multicentric prospective study on 133 patients with suspected acute coronary syndrome (ACS) and assessed the diagnostic and prognostic values of H-FABP in comparison with conventional cardiac markers. H-FABP and myoglobin showed greater positive results when compared with CK-MB or troponin T. ROC curve analysis revealed that H-FABP had the highest sensitivities among all the studied biomarkers for identification of patients requiring emergency hospitalization, coronary angiography, and interventional therapy within 7 days and hence, H-FABP was the most reliable marker for the detection of ACS. They concluded that, in patients with chest pain at an emergency department, H-FABP can be an early diagnostic and prognostic biochemical marker, particularly within the first 6 hrs after the onset of symptoms.[38]

In the year 2003, Yoshihiko Seino et al. assessed the clinical utility of H-FABP in comparison with to myoglobin and troponin T, among 371 consecutive patients presenting with acute chest pain, suspected of AMI. 181 patients (49%) had a final diagnosis of AMI. Among the 68 patients who presented within 2 hrs after the onset of symptoms, 37 (54%) patients were finally found to be diagnosed with AMI. The sensitivity of the rapid H-FABP test was 89% (33/37), significantly higher than for troponin T (22% [8/37]; P <0.001) and myoglobin (38% [14/37]; P <0.001). However,

the specificity of troponin T (94% [29/31]) was significantly better than H-FABP (52% [16/31]; P=0.002) within 2 hours. In patients who presented within 2 hours of onset of chest pain, H-FABP had greater area under ROC curve than that for myoglobin (0.72 vs. 0.61, P=0.01). They concluded that, "novel whole blood rapid H-FABP test can be helpful in the early evaluation of patients presenting with acute chest pain".[39]

In the year 2004, Alansari SE and Croal BL aimed to evaluate and compare the diagnostic accuracy of the early markers H-FABP and myoglobin (in terms of area under ROC curves, sensitivity and specificity) to TnI in a pragmatic group of patients presenting with chest pain. Blood samples were collected from 302 patients presenting with chest pain both on admission and 12 hrs later. These were analysed for TnI, myoglobin and H-FABP. Standard clinical and electrocardiographic data were also obtained. Median time from onset of symptoms to admission was 5.0 hrs (IQR 3.0-12 hrs). The area under ROC curve of cTnI for the diagnosis of MI was greater than both H-FABP and myoglobin. Both myoglobin and H-FABP also performed poorly in those patients who were TnI negative on admission. They concluded that, "myoglobin and H-FABP provide little clinical value when measured on admission in patients presenting with chest pain".[40]

In the year 2005, Cangel P Y Chan et al. presented a general review on the current status of different types of FABPs for the identification of tissue injury in patients with myocardial injury, brain injury and also in athletes or horses with skeletal muscle injury. FABP is a promising marker for the early detection of tissue injury. FABP is a small protein (15 kD) appears earlier in the blood than large proteins after cell damage.

Because of its characteristics such as high cytoplasmic concentration in the tissues and very low normal values in the blood provide the possibility of a rapid rise above the reference values, and thus can act as early indicator of the tissue injury. To take full advantage of the favourable characteristics of the early marker FABP, rapid analysis is a crucial factor. In this review, the development of an immunoassay for the quantification of FABP in buffer, plasma or whole blood was explained. The usefulness of different FABP immunosensors and immunotests were described. The feasibility of these immunoassays for their use in routine clinical practice and in emergency situations was also discussed. Nowadays, the improved automated immunoassays such as microparticle-enhanced turbidimetric immunoassay, and less time-consuming bedside immunosensors and immunotests such as one-step FABP lateral flow immunotest, have been developed. With these point-of-care tests, FABP is a potential marker of an early tissue injury, for many clinical uses.[41]

In the year 2005, Junnichi Ishii et al. compared the prognostic value of measurement of serum H-FABP concentration with that of cTnT within 6 hrs after the onset of chest pain among 241 (73.5%) AMI patients, 154 (47.0%) STEMI patients, and 287 (87.5%) patients who underwent emergency coronary angiography within 24 hrs after admission. During the 6-month follow-up period, 15 cardiac deaths and 10 subsequent nonfatal AMIs were observed. “Stepwise multivariate analyses of clinical, electrocardiographic, and biochemical variables revealed that increased H-FABP (above the median of 9.8 μg/L), but not increased cTnT (above the median of 0.02 μg/L), was independently associated with cardiac events in all patients [relative risk (RR) = 8.96; P = 0.0004], the subgroup of patients with ST-segment elevation myocardial infarction (RR =

11.3; P = 0.02), and the subgroup of patients with unstable angina and non-ST segment elevation myocardial infarction (RR = 8.31; P = 0.007)". H-FABP had greater area under the ROC curve than cTnT (0.711 vs 0.578; P=0.08), suggesting that serum H-FABP concentrations have a better predictive capacity for cardiac events than cTnT. They concluded that, serum H-FABP is a potential independent predictor of cardiac events within 6 months of patient admission and may provide prognostic information superior to cTnT in the early hours of ACS.[42]

In the year 2006, Daigo Nagahara et al. studied 74 patients with suspected acute coronary syndrome in the emergency department. Serum concentrations of CK-MB, cTnT, H-FABP, and myoglobin were measured in blood samples, and also myocardial perfusion and metabolic defects were scintigraphically quantified in all the patients soon after arrival. "CK-MB and cTnT had higher specificities (73 – 100%) but significantly lower positive rates (22 – 27%) than the others (61–68%). H-FABP and myoglobin (75 – 80%) had greater sensitivities than those of CK-MB and cTnT (29 – 35%). The biomarkers had high positive predictive values for detecting the defects (80 – 100%) but low negative predictive values (15 – 41%). Among the cardiac biomarkers, cTnT peak concentrations most closely correlated with scintigraphically detected cardiac abnormalities". Hence authors concluded that, "H-FABP can contribute to early detection of myocardial injury and cTnT is most likely to correlate with injured myocardial mass. The differential features of biomarkers are complementary for the detection of myocardial damage in patients with acute chest pain presenting at an emergency room".[43]

In the year 2006, Michelle O'Donoghue et al. estimated serum H-FABP concentration in total of 2287 patients with acute coronary syndromes from the OPUS-TIMI 16 trial and assessed usefulness of H-FABP in the assessment of prognosis of "acute coronary syndromes". "Serum H-FABP concentrations were significantly elevated (>8 ng/mL) in 332 patients (14.5%). During the 10-month follow-up period, patients with increased H-FABP levels were more likely to suffer death [hazard ratio (HR), 4.1; 95% CI, 2.6 - 6.5], recurrent myocardial infarction [HR, 1.6; 95% CI, 1.0 - 2.5], congestive heart failure [HR, 4.5; 95% CI, 2.6 - 7.8], or the composite of these end points [HR, 2.6; 95% CI, 1.9 - 3.5]. H-FABP predicted the risk of the composite end point both in patients who were troponin I negative (HR, 2.1; 95% CI, 1.3 to 3.4) and in those who were troponin I positive (HR, 3.3; 95% CI, 2.0 to 5.3). In a Cox proportional-hazards model that adjusted for baseline variables, including demographics, clinical characteristics, creatinine clearance, ST deviation, index diagnosis, and troponin I, elevated H-FABP remained a significant predictor of the composite end point (HR, 1.9; 95% CI, 1.3 to 2.7), as well as the individual end points of death (HR, 2.7; 95% CI, 1.5 to 4.9) and CHF (HR, 2.4; 95% CI, 1.2 to 5.0). In a multimarker approach, differential properties of H-FABP, troponin I, and B-type natriuretic peptide provided complementary information". The authors concluded that, "in patients presenting across the spectrum of acute coronary syndromes, elevation of H-FABP is associated with an increased risk of death and major cardiac events and is an independent of other established clinical risk predictors and biomarkers".[44]

In the year 2006, Ozcan Ruzgar et al. measured serum levels of H-FABP, CK-MB, and cTnT in 40 consecutive patients, of which 26 patients with onset of chest pain

within 6 hrs and 14 patients with onset of chest pain between 6 – 24 hrs. In 0 – 6 hr group, H-FABP (95%) had significantly higher sensitivity, followed by CK-MB (76%), and cTnT (38%) (P = 0.014). During 6 – 24 hr time period, cTnT (100%) had higher sensitivity followed by H-FABP (91%) and CK-MB (90%), but the difference was statistically insignificant. At the 24th hour, cTnT (100%) and CK-MB (90%) were more sensitive than H-FABP (27.3%) for the whole group (P = 0.002). "In the first group, H-FABP positivity was slightly but insignificantly higher in patients with two- and three-vessel disease compared with those with one-vessel disease (60.7% and 33.3%, P = 0.19) and in the same group, patients who underwent primary coronary intervention had a significantly higher H-FABP positivity than others (80%, 32%, P = 0.02). Within 6–24 hrs of chest pain, H-FABP positivity was 80% in patients with one-vessel disease and 71.4% in patients with two- and three-vessel disease (P = 0.69). Within 6–24 h, positivity of H-FABP reached a peak value of 100% in patients who underwent primary coronary intervention, while H-FABP was positive in 60% of the others (P < 0.001)". They conclude that, "within the 6 hrs of acute coronary syndrome, H-FABP seems to be a more sensitive biochemical marker than cTnT in the early detection of ischemic myocardial necrosis. But after the first 6 hrs of the onset of chest pain the sensitivity of H-FABP decreases, and this marker should not be used alone in patients admitted 24 hrs after the onset of chest pain".[45]

In the year 2007, P. MAD et al. "evaluated the utility of H-FABP for early diagnosis of AMI. Consecutive patients presenting with chest pain or dyspnoea within 24 hrs of symptom onset to the emergency department were included in the study. At presentation, the H-FABP test result was compared to the standard diagnostic work-up,

including repeated ECG and cTnT measurements. Sensitivity analysis was performed for inconclusive tests. They enrolled 280 patients presenting to hospital with a median symptom onset of 3 hrs (IQR 2–6 hrs): 109 (39%) had AMI. At presentation, H-FABP had a sensitivity of 69% (95% CI 59–77) and specificity of 74% (95% CI 66–80); 45 tests were false-positive and 34 were false-negative. Omitting inconclusive tests increased sensitivity and specificity only slightly. AMI was identified significantly earlier by H-FABP than by cTnT (24 vs. 8 patients, p = 0.005). They concluded that, Although H-FABP can help to detect myocardial damage at an early stage in patients with chest pain or dyspnoea, it appears unsuitable as a stand-alone test for ruling out AMI".[46]

In the year 2007, Hatice Pasaoglu et al. "investigated the clinical implications of serum H-FABP compared to myoglobin, cTnI, and CK-MB in patients with early phase AMI. Serum concentrations of H-FABP, myoglobin, cTnI, and CK-MB were measured in 21 AMI patients and 44 non-AMI controls. 3 blood samples were collected from each participant at 1-2, 3, and 6 hrs after the onset chest pain. The samples were compared to those of 20 age-matched healthy subjects. All the patients and healthy subjects had normal renal function. At 1-2, 3, and 6 hrs after the onset of AMI, similar to myoglobin, the diagnostic sensitivity and specificity of H-FABP were higher than those of cTnI and CK-MB. Greater receiver operating characteristic (ROC) curve areas for the diagnosis of MI, by both sets of criteria, were obtained for H-FABP and myoglobin compared to both cTnI and CK-MB. They concluded that, H-FABP and myoglobin are reliable biochemical markers for super-acute phase AMI and the changes in their serum concentrations have clinical significance in the diagnosis of AMI".[47]

In the year 2008, Łukasz Figiel et al. "evaluated the diagnostic value of early measurements of H-FABP and other markers of necrosis (cTnT, CK-MB, CK-MB mass) in a group of 100 patients with an acute coronary syndrome (ACS) without persistent ST segment elevation (NSTE ACS). During admission and after 3 and 6 hours, patients had measured a panel of conventional biomarkers as well as quantitative measurements of H-FABP (on admission and 3 hours later) using Cardio Detect med. The ultimate diagnosis of infarction (NSTEMI) was confirmed in case of a second (6 hrs after admission) positive quantitative result of cardiac troponin. Non-ST segment elevation MI was finally diagnosed in 56 patients. Results revealed that H-FABP was superior to other parameters, when measured on admission, and was characterised by 94.7% sensitivity, 100% specificity, 100% positive predictive value, 93.4% negative predictive value and 97% accuracy. Other biomarkers had lower sensitivity – 70.1% for CK-MB mass, 66.7% for CK-MB, 64.9% for cTnT, whereas their specificity was 97.6% for CK-MB mass, 93% for CK-MB and 100% for cTnT. They concluded that, qualitative H-FABP test (Cardio Detect med) showed excellent sensitivity, higher than measurements of CK-MB mass, CK-MB, and cTnT on hospital admission, and high specificity in the patient group with NSTEACS. The H-FABP seems to be an excellent biochemical cardiac marker for diagnosing NSTEMI, especially in its early phase, allowing exclusion of myocardial necrosis".[48]

In the year 2010, Yeongsic Kim et al. "compared an automated quantitative H-FABP assay with other cardiac-marker assays to examine its usefulness as an early diagnostic marker of AMI. Serum samples for cTnT, CK-MB, myoglobin, and H-FABP measurement were obtained from 64 patients with AMI and 53 controls. 2 immunoassays

were used for the measurement of H-FABP, the H-FABP enzyme-linked immunosorbent assay (ELISA; Biocheck, Foster City, CA) and the H-FABP latex turbidimetric immunoassay (LTIA; HBI, Anyang, Korea). Sensitivities of assays for cTnT, CK-MB, myoglobin, H-FABP (by ELISA), H-FABP (by LTIA), and electrocardiogram (ECG) for the diagnosis of AMI at hospital admission were 39.1%, 59.4%, 64.1%, 68.7%, 70.3%, and 54.7%, respectively. Respective Specificities of cTnT, CK-MB, myoglobin, H-FABP (by ELISA), H-FABP (by LTIA), and ECG were 98.1%, 71.7%, 81.1%, 77.4%, 90.6%, and 92.5%. They concluded that, the automated H-FABP (by LTIA) is superior to cTnT, CK-MB, myoglobin, and H-FABP (by ELISA) tests for the diagnosis of AMI in patients admitted within 4 hours from the onset of chest pain".[49]

In the year 2010, Hafidh A Alhadi et al. "evaluated the diagnostic value of H-FABP in patients with acute chest pain and compared it with standard cardiac markers. Serum cTnI, cTnT, CK-MB mass, myoglobin, and H-FABP were determined in 100 consecutive patients admitted with acute chest pain suggestive of acute coronary syndromes (ACS) at presentation and 2, 4, 8–10, and 16–24 hours after presentation. Results revealed that, H-FABP peak concentration occurred at 8 hours after symptoms onset and was the most sensitive early marker with 79.9% and 98% of patients with AMI identified at presentation and 2 hours after presentation respectively. The sensitivity of all other cardiac markers (CK-MB mass, cTnI, cTnT, and myoglobin) at presentation was < 62%. The negative predictive value of H-FABP (94%) was also superior to other markers within the first 2 hours of presentation. Myoglobin was the second most sensitive early marker at presentation. Peak sensitivity of cTnI, CK-MB mass, and cTnT were present at 4, 8–10, and 8–10 hours respectively after presentation. Conclusion: Combined

measurement of H-FABP and cTnI on two occasions during the first 8 hours after symptom onset was sufficiently sensitive and specific for the early diagnosis of most patients with acute MI and may provide advantages over other cardiac marker combinations".[50]

In the year 2010, Murat Orak et al. studied the effectiveness of the H-FABP in the early diagnosis of acute coronary syndrome (ACS) in patients admitted to emergency service (ES) within 6 hours of onset of chest pain. A total of 83 patients admitted with chest pain to our ES were included in this study. The patients were divided into 2 groups: "first group included 65 (78.3%) patients with a diagnosed ACS and second group included 18 (21.6%) non cardiac related chest pain patients". The above two groups were further divided into 2 groups as those admitted between 0 to 3 hours and 3 to 6 hours of onset of chest pain. Peripheral venous blood samples were obtained from all patients for H-FABP, troponin I, and CK-MB serum concentration measurements. The mean H-FABP level for the patients in the control group was 0.86 ± 0.54 ng/mL. There was a statistically significant difference between the ACS and control groups for the means of cardiac markers for CK-MB (P = .000) and H-FABP (P = .000), whereas no difference was observed for troponin I (P = .013). H-FABP sensitivity for diagnosis of ACS was found to be 98% and specificity was 71%; CK-MB sensitivity was 86% and specificity was 52%; and troponin I sensitivity was 77% and specificity was 20%. They concluded that, for patients admitted with chest pain to emergency service, H-FABP was found to be more sensitive and specific than troponin I and CK-MB in the early diagnosis of ACS.[51]

In the year 2011, Łukasz Figiel et al. "evaluated and compared the qualitative POCTs detecting H-FABP and glycogen phosphorylase – BB isoenzyme (GP-BB) in patients with an acute coronary syndrome (ACS). They studied 52 patients with chest pain lasting less than 6 hours and persistent ST-segment elevation suggestive of ACS. On admission, both H-FABP and GP-BB tests were performed using POCT devices. The study population was divided into two groups, one with chest pain lasting < 3 hrs (n = 20) and the other between 4–6 hrs (n = 32). The sensitivity of H-FABP (84%) was superior when compared to the other biomarkers, GP-BB and cTnT, which had sensitivity of 64% and 50%, respectively. Comparison of typical parameters of the diagnostic value of a test (sensitivity, predictive values and accuracy) in both time periods demonstrated that H-FABP was superior to GP-BB. In particular, sensitivity and accuracy of H-FABP was excellent in the group of patients with chest pain lasting < 3 hrs, with sensitivity of 79% for H-FABP and only 47% for GP-BB. Sensitivity and accuracy of cTnT were significantly lower (32% and 35%, respectively). They concluded that, the H-FABP seems to be an excellent early biomarker of cardiac necrosis in the group of patients with chest pain lasting < 3 hrs".[52]

In the year 2011, Kyung Su Kim et al. compared the diagnostic value of H-FABP when used in combination with cTnI with conventional cardiac markers for the diagnosis of AMI during the early hours. Blood samples were collected from consecutive patients with acute chest pain suggestive of AMI in the emergency department, and H-FABP, CK-MB, cTnI and myoglobin concentrations were estimated. Serial cTnI values were considered for the final diagnosis of MI. 76 patients out of 170 study participants were diagnosed as having MI. They assessed the adjunctive role of studied biomarkers by

comparing the logistic regression models predicting MI using their area under ROC curve and bayesian information criterion (BIC). "The area under ROC curves of cTnI, H-FABP, myoglobin, and CK-MB were 0.863, 0.827, 0.784, and 0.772, respectively. A logistic regression model using a combination of cTnI (P = 0.001) and H-FABP (P < 0.001) had the highest AUC (0.900) and the best fit as determined by BIC. Sensitivity, specificity, positive and negative likelihood ratios of this model at 30% probability were 81.6%, 80.9%, 4.26, and 0.23, respectively". The authors concluded that, "H-FABP had a better diagnostic value than myoglobin and CK-MB and it can be used as an adjunct to cTnI for the early diagnosis of MI".[53]

In the year 2012, C. Geraldine McMahon et al. "evaluated the diagnostic efficacy of H-FABP, cTnI, CK-MB, and myoglobin— for the early detection of AMI among patients who presented to the emergency department with chest pain. These cardiac biomarkers were assayed at serial time points in total of 2924 venous blood samples from 1128 study patients. H-FABP had the highest sensitivity in comparison with other cardiac markers at 0 to 3 hours (64.3%) and 3 to 6 hours (85.3%) after chest pain onset. Further, the combination of cTnI with H-FABP measurement increased the sensitivity to 71.4% at 0 to 3 hours and 88.2% at 3 to 6 hours. ROC curves demonstrated that H-FABP had the greatest diagnostic ability with highest area under the curve of 0.841 at 0 to 3 hours and of 0.894 at 3 to 6 hours. The specificity was also high for the combination of H-FABP with cTnI at these time points. The negative predictive value of H-FABP was highest of all the individual markers: 0 to 3 hours (93%) and 3 to 6 hours (97%). Again, the combined measurement of cTnI and H-FABP increased the negative predictive values to 94% at 0 to 3 hours, 98% at 3 to 6 hours, and 99% at 6 to 12 hours. The authors

concluded that, measurement of both H-FABP and cTnI levels is a reliable diagnostic tool for the early diagnosis of myocardial infarction / acute coronary syndrome and also a valuable rule-out test for patients presenting at 3 to 6 hours after the onset of chest pain".[54]

In the year 2012, Yonathan Freund et al. "assessed the incremental value of H-FABP to cardiac troponin for a rapid rule out of AMI, according to the pretest probability (PTP) of AMI. In consecutive patients presenting to emergency department with chest pain of less than 6 hours duration suggestive of AMI, H-FABP levels were measured, blinded to the emergency department physicians, and they were asked to quote the PTP of AMI. The diagnosis was done by 2 independent experts, blind to the H-FABP level. Out of 317 patients (mean age of 57 years) included, 149 had (47%) low, 117 (37%) moderate, and 51 (16%) high PTP. The final diagnosis was AMI in 45 patients (14%), including 16 STEMIs (5%). The negative predictive value for diagnostic elimination of AMI of H-FABP less than 3µg/L, combined with a negative cTnI was not higher than that of cTnI alone [96% (95% confidence interval, 93%-98%) vs 95% (93%-98%)], regardless of the PTP. Even in the low-PTP group, authors did not demonstrate a significant improvement in negative predictive value with the addition of H-FABP, compared with that of cTnI alone [100% (97%-100%) vs 99% (96%-100%)]".[55]

In the year 2012, Ibrahim Elmadbouh et al. "determined the efficacy of H-FABP in comparison with myoglobin, CK-MB and cTn-I in the early diagnosis of AMI in patients presenting with acute chest pain. The patients were classified as AMI (n=22), unstable angina (UA, n= 20) and non-cardiac chest pain (NCCP, n=15) within 3 hrs and 6

hrs of acute chest pain according to the American College / European Society of Cardiology; and normal healthy subjects (controls, n= 10). Blood H-FABP levels were measured by ELISA and compared with that of cTn-I, CK-MB and myoglobin in all subjects. Serum H-FABP, myoglobin, CK-MB and cTn-I were significantly higher in AMI than the UA, NCCP (non-AMI) and control groups within 6 hrs. However, Serum H-FABP, myoglobin and CK-MB were significantly elevated within 0–3 hrs and extend more within 3–6 hrs in AMI versus non-AMI. The cutoff value of H-FABP in AMI was 21.85 ng/ml within 3 hrs, and had diagnostic sensitivity (81.8%) equal to that of CK-MB and cTn-I but superior to that of myoglobin (72.7%). However, H-FABP had higher specificity (88.2%) equal to that of myoglobin but superior to that of CK-MB and cTn-I. This trend extends to within 6 hrs as well. Moreover, ROC curve areas for H-FABP were significantly higher ($p< 0.05$) than other biomarkers <6 hrs after the onset of chest pain. Hence authors concluded that, H-FABP can be used as a sensitive biomarker for myocardial injury in early stage".[56]

In the year 2013, Carless DR et al. "evaluated the Randox Laboratories immuno-turbidimetric assay on a Siemens Advia 1800 analyzer. The assay uses latex particles coated with mouse monoclonal anti-H-FABP antibodies to generate turbidity. They used redundant patient samples and pools to assess precision, functional sensitivity, limit of detection, linearity, recovery of recombinant H-FABP and interference. They evaluated the 99th percentile values and compared H-FABP with troponin-I in samples routinely received from chest pain patient samples. Precision was typically < 10% and 12.5% at all concentrations for within and between batches. The assay had a functional sensitivity of 2.4 μg/L. The linearity range on dilution for the assay was 2.76-115 μg/L. Recovery of

recombinant H-FABP was approximately 20-25%. There was no interference observed with haemoglobin concentrations <1.5 g/L, bilirubin < 250 μg/L and triacylglycerol < 5 mmol/L or rheumatoid factor. The 99th percentile value in a reference population with eGFR > 60mL/min/1.73m^2 was 9.1 μg/L with no significant gender difference. H-FABP was measured in 1310 routine clinical samples received for troponin-I measurement. Area under ROC curve of H-FABP was 0.82 using Siemens TnI > 50 ng/L as an indicator of myocardial damage. They concluded that, the immunoturbidimetric H-FABP assay is robustly designed and shows good analytical performance. It is therefore well suited for routine use in clinical laboratory".[57]

In the year 2013, Jan F. C. Glatz et al. assessed the plasma H-FABP reference values using a H-FABP immunoassay with improved analytical performance in a relatively large group (n = 443) of healthy individuals aged between 18 to 69 years. Authors found that, Mean (±SD) plasma H-FABP concentration was 1.7 ± 0.9 ng/mL, with somewhat higher values found in males than in females. Plasma H-FABP concentration also increases with age, as previously reported. The 99th percentile H-FABP level was found to be 5.6 ng/mL for subjects aged 41 - 69 years.[58]

In the year 2013, M.H.E. Bruins Slot et al. did a study to determine the diagnostic accuracy of a rapid H-FABP test in patients suspected of acute coronary syndrome (ACS) in primary care. General practitioners included 298 patients suspected of ACS. In all patients, whether referred to hospital or not, ECG and cardiac biomarker testing was performed. ACS was determined in accordance with international guidelines. The H-FABP bedside test was performed within 24 hrs (median 3.1, IQR 1.5 to 7.1) after

symptom onset. The positive predictive value (PPV) of H-FABP was 65% (95% confidence interval (CI) 50–78). The negative predictive value (NPV) was 85% (95% CI 80–88). Sensitivity was 39% (29–51%) and specificity 94% (90–96%). Within 6 hrs after symptom onset, the PPV was 72% (55–84) and the NPV was 83% (77–88), sensitivity 43% (31–57%) and specificity 94% (89–97%). Adding the H-FABP test to a diagnostic model for ACS led to an increase in the area under the ROC curve from 0.66 (95% CI 0.58–0.73) to 0.75 (95% CI 0.68–0.82). They concluded that, The H-FABP rapid test provides modest additional diagnostic certainty in primary care. It cannot be used to safely rule out ACS. The test can only be used safely in patients otherwise NOT referred to hospital by the general practitioner, as an extra precaution not to miss ACS (‘rule in’).[59]

In the year 2014, Priscilla Abraham Chandran et al. evaluated the diagnostic performance of serum HFABP in comparison to cardiac cTnT and TnI in 33 patients admitted with chest pain, diagnosed as NSTE-ACS (non ST elevation acute coronary syndrome) and 22 healthy controls. Area under the ROC curve (AUC) was highest for H-FABP (AUC 0.79; 95% CI 0.66 - 0.89) versus cTnI (AUC 0.73; 95% CI 0.59 - 0.84) and cTnT (AUC 0.71; 95% CI 0.57 - 0.83).The H-FABP level above 6.5 ng/mL showed 56.7% (CI 37.4 - 74.5) sensitivity, 0.5 (95% CI 0.3 - 0.7) negative likelihood ratio (LR), 100% (CI 84.6 - 100.0) specificity, and 100% (CI 79.4 - 100.0) positive predictive value (PPV), 62.9% (CI 44.9 - 78.5) negative predictive value (NPV). cTnI level above 0.009 µg/L had 40% (CI 22.7 - 59.4) sensitivity, 0.6 (95% CI 0.4 - 0.8) LR, 100% (CI 84.6 - 100.0) specificity, 100% (CI 73.5 - 100.0) PPV, and 55% (CI 38.5 - 70.7) NPV. cTnT showed 46.7% (CI 28.3 - 65.7) sensitivity, 0.5 (95% CI 0.4 - 0.7) LR, 100% (CI 84.6 -

100.0) specificity, 100% (CI 76.8 - 100.0) PPV, and 57.9% (CI 40.8 - 73.7) NPV at level above 9 µg/L. LR were 12.5 (95% CI 1.8 - 86.8), 1.7 (95% CI 1.0 - 3.0), and 1.2 (95% CI 0.8 - 1.9) for H-FABP, cTnI, and cTnT respectively. They concluded that, measurement of H-FABP is a valuable tool in the early diagnosis of patients with chest pain (6.8 hrs) and seems to be a preferred biomarker in the differential diagnosis of NSTE-ACS. More studies are needed to determine whether serum H-FABP further improves diagnostic performance.[60]

In the year 2014, Robert TA Willemsen and coworkers tested the value of H-FABP alone or with routinely used biomarkers such as myoglobin, CK-MB, and cTn I in patients who admitted to emergency department (ED) with complaint of chest pain suggestive of acute coronary syndrome. Patients who were admitted within first 48 hours of chest pain suggestive of ACS were included in the study. H-FAPB values were positive in 15.2% patients. When H-FABP was added to routinely used biomarkers in the diagnosis of ACS, there was increase in the sensitivity, specificity, PPV and NPV values. However, this increase was not statistically significant. They concluded that, H-FABP did not provide any significant change in early diagnosis and exclusion of ACS diagnosis when used either alone or in combination with routinely used biomarkers.[61]

In the same year, Simona Da Molin and his co-investigators verified the analytical performance of immuno-turbidimetric assay for H-FABP claimed by Randox Laboratories, on Roche Cobas 6000 clinical chemistry platform in their laboratory, and defined their own 99th percentile upper reference limit for H-FABP. For the verification of method performances, they used pools of spared patient samples from routine and two

levels of quality control material, while samples for the reference value study were collected from 545 blood donors. As per the CLSI guidelines, they verified limit of blank (LOB), limit of detection (LOD), limit of quantitation (LOQ), repeatability and within-laboratory precision, trueness, linearity, and the stability of H-FABP in EDTA over 24 hrs. The LOQ (3.19 μg/L) was verified with a CV% of 10.4. The precision was verified for the low (mean 5.88 μg/L, CV = 6.7%), the medium (mean 45.28 μg/L, CV = 3.0%), and the high concentration (mean 88.81 μg/L, CV= 4.0%). The trueness was verified as well as the linearity over the indicated measurement interval of 0.747–120 μg/L. The H-FABP in EDTA samples is stable throughout 24 hrs both at room temperature and at 4 °C. The H-FABP 99th percentile upper reference limit for all subjects (3.60 μg/L, 95% CI 3.51–3.77) is more appropriate than gender-specific ones that are not statistically different.[62]

In the same year, Vupputuri A prospectively investigated the usefulness of H-FABP determination for the evaluation of acute chest pain in patients arriving at the emergency department. Fifty-four patients presenting with acute ischemic chest pain were enrolled for the study. Serum H-FABP concentration was estimated at admission using latex-enhanced immunoturbidimetric assay. Serial cardiac troponin I (cTnI), creatinine kinase-MB (CK-MB) determination, ischemia workup with stress testing, and/or coronary angiogram were performed according to standard protocols. The sensitivity and specificity of H-FABP was 89.7% and 68%, for cTnI it was 62.1% and 100%, and for CK-MB it was 44.8% and 92%, respectively for diagnosis of AMI. The sensitivity of H-FABP was found to be far superior to initial cTnI and CK-MB, for those seen within 6 hrs (100% vs. 46.1%, 33% respectively). On further evaluation of patients

with positive H-FABP and negative cTnI, 71.4% of the patients had significant lesion on coronary angiogram, indicating ischemic cause of H-FABP elevation. Six patients with normal cTnI and CK-MB with high H-FABP had ST elevation on subsequent ECGs and were taken for primary angioplasty. They concluded that, H-FABP is a highly sensitive biomarker for the early diagnosis of AMI. H-FABP as early marker and cTnI as late marker would be the ideal combination to cover the complete diagnostic window for AMI.[63]

In the year 2016, Shama Prakash Kabekkodu and other 2 investigators in south India evaluated the role of H-FABP in early detection of AMI by comparing its sensitivity, specificity and predictive value with CK-MB and cTnI. 50 patients admitted with the diagnosis of AMI were categorised in to those coming to the hospital within four hours of symptom onset and those coming in between 4 to 12 hours. H-FABP was compared with those of cTnI and CK-MB tests. Among patients presenting within four hours of symptom onset, the sensitivity of H-FABP was 60% and was significantly higher than that of cTnI (18.8%) and CK-MB (12.5%). But specificity was only 23.53% and was less than that of cTnI (66.67%) and CK-MB (100%). In patients presenting during 4 to 12 hours of symptom onset, the sensitivity of H-FABP was 86.96% which was comparable to that of cTnI (90.9%) and CK-MB (77.3%). The specificity was 60% in the 4-12 hours group which was comparable to that of cTnI (50%) and CK-MB (50%). They concluded that, H-FABP is a sensitive biomarker for the diagnosis of AMI in the initial hours after symptom onset when the standard biomarkers may not be elevated, but it is less specific. During 4-12 hours of symptom onset it is as sensitive and specific as standard cardiac biomarkers troponin I and CK-MB. Due to these factors H-FABP can be

considered as a promising cardiac biomarker which can be used along with troponins and CK-MB at present.[64]

In the year 2017, Paul Collinson et al did a study to establish the analytical performance of H-FABP method suitable for routine clinical use and examined its role for the diagnosis of myocardial ischemia and myocardial infarction. Analyses of H-FABP were performed on an Advia 2400 (Siemens Healthcare Diagnostics). Imprecision, limit of detection (LOD), limit of blank (LOB), and linearity were assessed using standard methods. Clinical diagnostic performance was assessed using chest pain in patients, with a final diagnosis according to the universal definition of myocardial infarction with cardiac troponin I (cTnI) measured on the Siemens Advia Centaur (cTnI Ultra method, 99th percentile 50 ng/L, 10% CV 30 ng/L). Ischemia was detected using sampling pre- and postangioplasty. They found that, LOD and analytical imprecision exceeded the manufacturer's specification (LOD 1.128 μg/L, 20% CV 1.3 μg/L, 10% CV 2.75 μg/L). Clinical diagnostic efficiency was less than cTnI. Addition of HFABP to cTnI produced a modest increase in diagnostic sensitivity at a cost of significant loss of specificity. They concluded that, although the test had excellent analytical performance, it did not contribute to the clinical diagnosis of patients with chest pain. H-FABP appears to be a marker of myocardial infarction not myocardial ischemia.[65]

Chapter 4
Materials & Methods

MATERIALS AND METHODS

4.1 Study design

4.1.1 Scope of the study: The present study has been designed to evaluate the diagnostic value of novel biochemical marker "heart type fatty acid binding protein (H-FABP)" in the early detection of acute myocardial infarction particularly within 6 hours after the onset of chest pain suggestive of ischemic heart disease, in comparison with currently available conventional biomarkers such as "CK-MB, troponin I and myoglobin".

4.1.2 Study setting: The patients attending the emergency department and intensive cardiac care unit (ICCU), BLDE [Deemed to be University] Shri B M Patil Medical College, Hospital and Research Centre, Vijayapura, with the presenting complaint of acute chest pain of less than 6 hours duration suspected of AMI.

4.1.3 Study period: From May 2013 to June 2014.

4.1.4 Sample size: The pooled estimates from studies carried out in 1990s up to 2002 in India shows the prevalence rate of CHD in urban areas as 6.4 percent and 2.5 percent in rural areas. Taking the urban population 30% and rural population 70% of total, prevalence rate (urban & rural) = (64.37 x 0.3) + (25.27 x 0.7) = 37.0 per 1000 (3.7%).[66]

The confidence interval of 95% and margin of error of ±5 are considered.

The sample size is calculated using the following formula.

$$n = (1.96)^2 \times p \times (1-p) / d^2$$

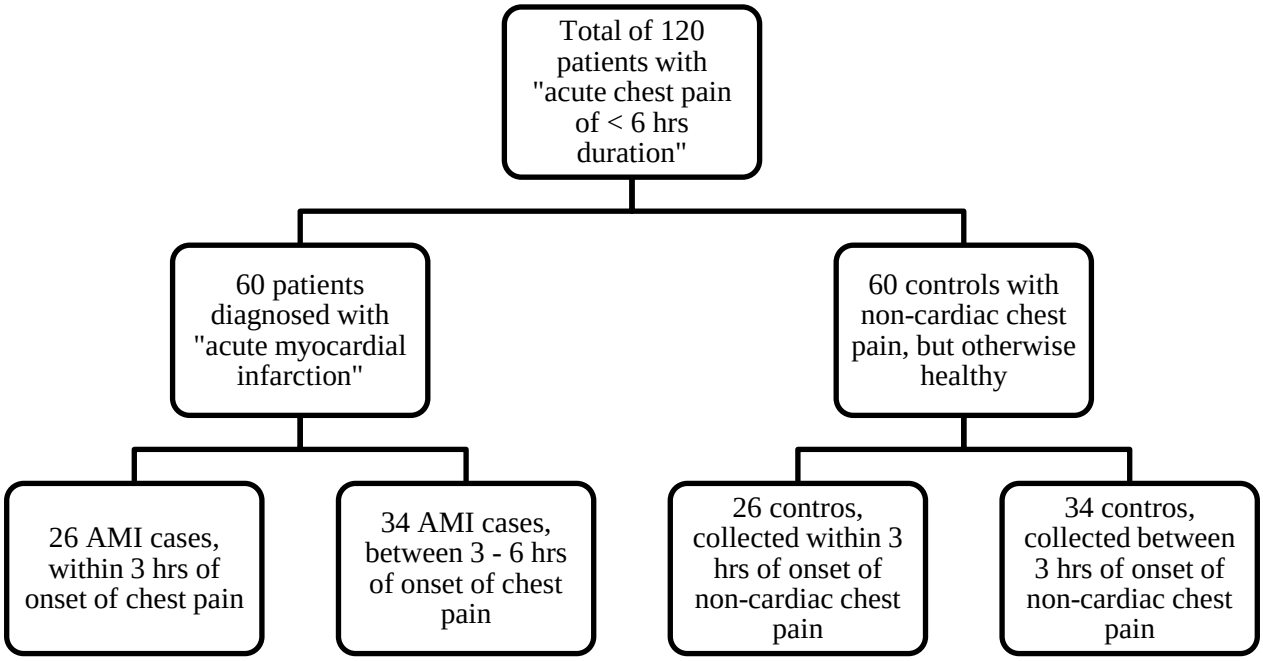
n = Sample size; p = Prevalence; d = margin of error.

Using this formula the minimum sample size is 55.

4.1.5 Study type: It is a cross sectional, hospital based study.

4.1.6 Study subjects: A total of 120 patients, aged between 30 – 70 years, attending the emergency department and intensive cardiac care unit (ICCU), BLDE [Deemed to be University] Shri B M Patil Medical College, Hospital and Research Centre, Vijayapura, with the presenting complaint of acute chest pain of less than 6 hours duration suspected of AMI.

4.1.7 Study groups:



4.1.8 Inclusion criteria:

1. **Cases**: Patients aged between 30 – 70 years, visiting the cardiology OPD or casualty of Shri B M Patil Medical College, Hospital and Research Centre, Vijayapura, because of acute chest pain and/or dyspnoea and other symptoms strongly suggestive of myocardial infarction, within 6 hours of onset of symptoms

2. **Controls:** Age and sex matched non-cardiac chest pain patients, within 6 hours of onset of chest pain, without any major illness.

4.1.9 Exclusion criteria:

1. Patients arriving to hospital after 6 hours of onset of chest pain or other symptoms suggestive of myocardial infarction
2. Those with chronic muscle disease, renal disease or recent surgery
3. Those receiving direct current shocks and
4. Who underwent Percutaneous Transluminal Coronary Angioplasty (PTCA) or Coronary Artery Bypass Graft (CABG) procedures within the last 30 days
5. Those patients not willing to give consent

4.2 Ethical aspects

4.3.1 Informed consent: all the subjects were explained very clearly about the purpose and outcomes of the present study in their own language and a written informed consent was obtained from them.

4.3.2 Institutional ethical clearance: The study was approved by the Institutional Ethical Clearance Committee, BLDE [Deemed to be University] Shri B M Patil Medical College, Hospital and Research Centre, Vijayapura.

4.3.3 Ethical standards: All the procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

4.3 Study protocol

4.3.1 Method of collection of data: Patients presenting with acute chest pain less than 6 hours duration suspected of AMI were enrolled consecutively in the study. An informed consent was obtained from each participant before including in the study. Complete clinical history including basic demographic data, presenting symptoms, past medical history such as diabetes mellitus, hypertension, previous ischemic event, renal disease etc, and general clinical examination findings, ECG findings, Echocardiography and routine laboratory investigations such as complete blood count, random blood glucose, renal function tests, lipid profile and troponin I findings were documented using a predefined protocol. Diagnosis of AMI was done according to "European Society of Cardiology/ American College of Cardiology Committee (ESC/ACCC) Criteria". AMI was defined as "detection of initial or 6 hrs cardiac Troponin I > 0.1 ng/ml together with evidence of myocardial ischemia with at least one of the following: 1. Symptoms of ischemia; 2. ECG changes indicative of new ischemia (new ST-T changes); 3. Development of pathological Q waves in the ECG; 4. Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality".[11]

The cases and controls were further divided into two subgroups depending on the time since the onset of chest pain as the subjects within 3 hrs of onset of chest pain and those between 3 and 6 hrs of onset of chest pain.

4.3.2 Collection of blood samples: From all the cases and controls, about 5 mL of blood sample was drawn under all aseptic precautions from a large peripheral vein by using a sterile disposable syringe and collected in a sterile plain bulb. The

blood was allowed to clot for about 30-45 minutes and then the serum was separated by centrifugation at 5000 rpm for 15 minutes and kept at -20^0 C in order to secure accurate and reproducible results. The serum samples were analyzed for the following study parameters as early as possible.

4.3.3 Biochemical analysis: In the present study, the following parameters were estimated in the serum.

1. Heart-type fatty acid binding protein
2. CK-MB
3. Troponin I
4. Myoglobin

Serum H-FABP levels were measured by automated immuno-turbidimetric method (Reagent kit from Randox Laboratories Limited, County Antrim, United Kingdom) on Roche cobas c 311 fully automated Biochemistry analyzer (Roche Diagnostic Limited, Rotkreuz, Switzerland). Serum CK-MB activity was measured by Immuno-inhibition method using Stat Fax 3300 Biochemistry analyzer (Awareness Technology Inc., Florida, USA). Serum troponin-I and myoglobin levels were measured by chemiluminescence immunoassay on Abbott Architect c4000 analyzer (Abbott Laboratories, Illinois, USA).

4.3.3.4 Estimation of serum 'heart-type fatty acid binding protein'

Method: Immuno-turbidimetric method.[67,68]

Principle: "Sample is made to react with a buffer and anti-H-FABP coated latex. The formation of the antibody-antigen complex during the reaction results in an increase in turbidity, the extent of which is measured as the amount of light absorbed at 700 nm. By

constructing a standard curve from the absorbance of the standards, H-FABP concentration in the sample can be determined".

Sample collection and preparation: Normal procedures for collecting and storing serum may be used for samples to be analysed by this method. If not analysed immediately, serum or plasma should be stored at -20°C.

Reagents composition:

1. R1: Buffer Sodium Azide < 0.1% w/v
2. R2: Antibody-latex Reagent: Mouse monoclonal anti-H-FABP antibodies

   Sodium Azide < 0.1% w/v

Procedure:

R1 160 µl + Sample 8 µl

↓

Incubation for 5 minutes

↓

R2 40 µl

↓

Incubation for 12 – 40 seconds (optimum)

↓

Read Absorbance A1 at 700 nm

↓

Incubation for 5 minutes after R2 addition

↓

Read Absorbance A2 at 700 nm

↓

H-FABP result reported

Reference values: < 6.32 ng/ml (99th percentile)

4.3.3.2 Estimation of serum creatinine kinase – MB isoenzyme activity

Method: Immuno-inhibition method[69,70]

Principle: The 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. CK-B activity is measured by the increasing rate of absorbance resulting from the following reactions.

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK (AMP,NAC)}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{Glucose} \xrightarrow{\text{Hexokinase}} \text{ADP} + \text{Glucose} - 6 - \text{P}$$

$$'\text{G} - 6 - \text{P}' + '\text{NADP}' + \text{H}_2\text{O} \xrightarrow{\text{G-6-P Dehydrogenase}} '\text{Gluconate} - 6 - \text{P}' + '\text{NADPH} + \text{H}^+$$

CK-MB activity is obtained by multiplying CK-B activity by 2.

Reagents composition:

CK-MB – R1:

1. 'Imidazole (pH 6.7) – 125 mmol/L'

2. ‘D-Glucose – 25 mmol/L’
3. ‘N-Acetyl-L-Cysteine – 25 mmol/L’
4. ‘Magnesium acetate – 12.5 mmol/L’
5. ‘NADP – 2.52 mmol/L’
6. ‘EDTA – 2.02 mmol/L’
7. ‘Hexokinase – ≥6800 U/L’
8. CK-MM.

CK-MB – R2:

1. Creatinine phosphate – 250 mmol/L
2. ADP – 15.2 mmol/L
3. AMP – 25 mmol/L
4. ‘Diadenosine pentaphosphate – 103 µmol/L’
5. ‘G-6-PDH – ≥8800 U/L’

Preparation of working reagent:

Mix 4 volume of Reagent 1 (R1) with 1 volume of Reagent 2 (R2)

General system parameters:

1. Mode of reaction – kinetic
2. Slope of reaction – Increasing
3. Wavelength – 340 nm
4. Temperature – 37^0 C
5. Factor – 8254
6. Linearity – 1032 U/L

7. Blank – DI water
8. Delay time – 300 seconds
9. No. of readings – 3
10. Interval – 60 seconds
11. Sample volume – 40 µL
12. Reagent volume – 1000 µL▲

Procedure:

SI. No.	**Reagent**	**Volume**
01.	Working reagent	1000 µL
02.	Sample	40 µL
Mix and incubate at 37^0 C for 5 minutes. Read the 'change in absorbance per minute' (▲OD/minute) during 3 minutes.		

Calculation:

"CK-MB activity (U/L) = (▲OD/min) X 8254"

Normal range: Up to 24 U/L.

4.3.3.4 Estimation of serum troponin I

Method: Chemiluminescence immunoassay[71]

Principle: "In the first step, sample, assay diluent and anti-troponin-I antibody-coated paramagnetic microparticles are combined. Troponin-I present in the sample binds to the anti-troponin-I coated microparticles. After incubation and wash, anti-troponin-I acridinium-labeled conjugate is added in the second step. Following another incubation

and wash, pre-trigger and trigger solutions are then added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of troponin-I in the sample and the RLUs detected by the ARCHITECT i* System optics. The concentration of troponin-I is read relative to a standard curve established with calibrators of known troponin-I concentrations".

Reagents composition:

ARCHITECT STAT Troponin-I Reagent Kit (2K41) (100 Tests):

- **Microparticles:** 1 or 4 Bottles (6.6 mL/27.0 mL) Anti-troponin-I (mouse, monoclonal) coated microparticles in TRIS buffer with protein (bovine and goat) stabilizers. Preservatives: antimicrobial agents.
- **Conjugate:** 1 or 4 Bottles (5.9 mL/26.3 mL) Anti-troponin-I (mouse, monoclonal) acridinium-labeled conjugate in MES buffer with protein (bovine) stabilizer. Preservative: ProClin 300.
- **Assay diluent:** 1 or 4 Bottles (10.0 mL/50.9 mL) Troponin-I Assay Diluent, containing protein (bovine and goat) stabilizers in phosphate buffer. Preservative: ProClin 300.
- **ARCHITECT i Pre-Trigger Solution:** 1.32% (w/v) hydrogen peroxide.
- **ARCHITECT i Trigger Solution:** 0.35 N sodium hydroxide.
- **ARCHITECT i Wash Buffer:** Phosphate buffered saline solution. Preservatives: antimicrobial agents.

Procedure:

Materials Required:

- 2K41 ARCHITECT STAT Troponin-I Reagent Kit

- ARCHITECT i System with STAT protocol capability
- 3K51 ARCHITECT i - US - Addition B
- 3K53 ARCHITECT i - WW (excluding US) - Addition B
- 2K41-01 ARCHITECT STAT Troponin-I Calibrators
- 2K41-10 ARCHITECT STAT Troponin-I Controls
- ARCHITECT I Pre-Trigger Solution
- ARCHITECT I Trigger Solution
- ARCHITECT I Wash Buffer
- ARCHITECT i Reaction vessels
- ARCHITECT i Sample cups
- ARCHITECT i Septum
- ARCHITECT i Replacement caps
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

Assay Procedure:

- Before loading the ARCHITECT STAT Troponin-I Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during the storage:
  - Invert the microparticle bottle 30 times.
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - If the microparticles do not resuspend, DO NOT USE.

- Once the microparticles have been resuspended, place a septum on the bottle.

- Order calibration, if necessary.
- Order tests.
- Load the ARCHITECT STAT Troponin-I Reagent Kit on the ARCHITECT i System with STAT protocol capability.
  - Verify that all necessary assay reagents are present. Ensure that septums are present on all reagent bottles.
- The minimum sample volume is calculated by the system and is printed on the Order list report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
  - Priority: 165 µL for the first ARCHITECT STAT Troponin-I test plus 115 µL for each additional ARCHITECT STAT Troponin-I test from the same sample cup.
  - ≤ 3 hours on board: 165 µL for the first ARCHITECT STAT Troponin−I test plus 115 µL for each additional ARCHITECT STAT Troponin-I test from the same sample cup.
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare calibrators and controls.
  - ARCHITECT STAT Troponin-I Calibrators and Controls should be mixed according to instructions in their respective package inserts.

- To obtain the recommended volume requirements for the ARCHITECT STAT Troponin-I Calibrators, hold the bottles vertically and dispense 9 drops of each calibrator into each respective sample cup. Dispense 165 µL of each control into each respective sample cup.

- Load samples.
- Press RUN.

Specimen Dilution Procedures

Specimens with a troponin-I value exceeding 50.00 ng/mL (50.00 µg/L) are flagged with the code ">50.00" and may be diluted with the Automated Dilution Procedure or the Manual Dilution Procedure.

Automated Dilution Protocol

- If using the Automated Dilution Protocol, the system performs a 1:9 dilution of the specimen and automatically calculates the concentration of the specimen before dilution and reports the result.
- Specimens with a troponin-I value exceeding 440.00 ng/mL (440.00 µg/L) are flagged with the code ">440.00" when run using the Automated Dilution Protocol. These specimens may be diluted by the following Manual Dilution Procedure.

Manual Dilution Procedure

- The suggested dilution for a troponin-I test is 1:20.
- Prior to diluting the specimen, dispense several drops of ARCHITECT STAT Troponin-I Calibrator A into a clean test tube for use in the next step.

- Add 10 µL of the patient specimen to 190 µL of ARCHITECT STAT Troponin-I Calibrator A.
- The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution. This will be the reported result. The concentration of the specimen diluted (before dilution factor is applied) should be 2.5 ng/mL (2.5 µg/L) or greater.

Calibration

- To perform an ARCHITECT STAT Troponin-I calibration, test the Calibrators A, B, C, D, E, and F in duplicate. A single sample of each ARCHITECT STAT Troponin-I Control level must be tested to evaluate the assay calibration. Ensure that assay control values are within the concentration ranges specified in the control package insert. Calibrators should be priority loaded.
- Calibration Range: 0.00 - 50.00 ng/mL (0.00 - 50.00 µg/L).
- Once an ARCHITECT STAT Troponin-I calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
    - A reagent kit with a new lot number is used.
    - Controls are out of range.

Quality Control Procedures:

- The recommended control requirement for the ARCHITECT STAT Troponin-I assay is a single sample of each control level to be tested once every 24 hours each day of use. If the quality control procedures in your laboratory require more

frequent use of controls to verify test results, follow your laboratory-specific procedures.

- The ARCHITECT STAT Troponin-I Control values must be within the acceptable ranges specified in the control package insert. If a control is out of its specified range, the associated test results are invalid and must be retested. Recalibration may be indicated.

Calculation

The ARCHITECT STAT Troponin-I assay uses a 4 Parameter Logistic Curve Fit (4PLC, Y-weighted) data reduction method to generate a calibration curve.

Expected Values:

- Males: < 0.032 µg/L
- Females: < 0.022 µg/L

4.3.3.4 Estimation of serum myoglobin

Method: Chemiluminescence immunoassay[72]

Principle: **"**In the first step, sample and anti-myoglobin coated paramagnetic microparticles are combined and incubated. Myoglobin present in the sample binds to the anti-myoglobin coated microparticles. After washing, anti-myoglobin acridinium-labeled conjugate is added in the second step. Following another incubation and wash, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of myoglobin in the sample and the RLUs".

Reagents:

ARCHITECT STAT Myoglobin Reagent Kit (2K43) (100 Tests)

- **Microparticles:** 1 or 4 Bottles (6.6 mL/27.0 mL) Anti-myoglobin (mouse, monoclonal) coated microparticles in TRIS buffer with protein (bovine) stabilizer. Preservative: antimicrobial agents.
- **Conjugate:** 1 or 4 Bottles (5.9 mL/26.3 mL) Anti-myoglobin (mouse, monoclonal) acridinium-labeled conjugate in MES buffer with protein (bovine) stabilizer. Preservative: ProClin 300.
- **Specimen diluents:** 1 or 4 Bottles (10.0 mL/50.9 mL) Myoglobin Specimen Diluent containing protein (bovine) stabilizer in TRIS buffer. Preservative: sodium azide.
- **Pre-Trigger Solution:** 1.32% (w/v) hydrogen peroxide.
- **Trigger Solution**: 0.35 N sodium hydroxide.
- **Wash Buffer:** Phosphate buffered saline solution. Preservative: antimicrobial agent".

Procedure:

Materials required:

- 2K43 ARCHITECT STAT Myoglobin Reagent Kit
- ARCHITECT i System with STAT protocol capability
- 3K51 ARCHITECT i – ASSAY CD-ROM – US – Addition B
- 3K53 ARCHITECT i - ASSAY CD-ROM - WW (excluding US) - Addition B
- 2K43-01 ARCHITECT STAT Myoglobin Calibrators
- 2K43-10 ARCHITECT STAT Myoglobin Controls
- ARCHITECT i Pre-Trigger Solution

- ARCHITECT i Trigger Solution
- ARCHITECT i Wash Buffer
- ARCHITECT i Reaction vessels
- ARCHITECT i Sample cups
- ARCHITECT i Septum
- ARCHITECT i Replacement caps
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

Assay Procedure:

- Before loading the ARCHITECT STAT Myoglobin Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment.
    - Invert the microparticle bottle 30 times.
    - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles remain adhered to the bottle, continue inverting the bottle until the microparticles have been completely resuspended.
    - If the microparticles do not resuspend, DO NOT USE.
    - Once the microparticles have been resuspended, remove and discard the cap. Wearing clean gloves, remove a septum from the bag. Squeeze the septum in half to confirm that the slits are open. Carefully snap the septum onto the top of the bottle.
- Order calibration, if necessary.
- Order tests.

- Load the ARCHITECT STAT Myoglobin Reagent Kit on the ARCHITECT i System with STAT protocol capability.
    - Verify that all necessary assay reagents are present. Ensure that septums are present on all reagent bottles.
- The minimum sample cup volume is calculated by the system and is printed on the Orderlist report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
    - Priority: 80 µL for the first ARCHITECT STAT Myoglobin test plus 30 µL for each additional ARCHITECT STAT Myoglobin test from the same sample cup.
    - ≤ 3 hours on board: 150 µL for the first ARCHITECT STAT Myoglobin test plus 30 µL for each additional ARCHITECT STAT Myoglobin test from the same sample cup.
    - To minimize the effects of evaporation, all samples (patient specimens, calibrators and controls) must be tested within 3 hours of being placed on board the ARCHITECT i System.
    - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare calibrators and controls.
    - ARCHITECT STAT Myoglobin Calibrators and Controls should be prepared according to their respective package inserts.

- To obtain the recommended volume requirements for the ARCHITECT STAT Myoglobin Calibrators, hold the bottles vertically and dispense 8 drops of each calibrator into each respective sample cup. Dispense 150 µL of each control into each respective sample cup.

- Load samples.
- Press RUN. The system performs the following functions:
  - Moves the sample to the aspiration point.
  - Loads a reaction vessel (RV) into the process path.
  - Aspirates and transfers sample into the RV.
  - Advances the RV one position and transfers microparticles into the RV.
  - Mixes, incubates, and washes the reaction mixture.
  - Adds conjugate to the RV.
  - Mixes, incubates, and washes the reaction mixture.
  - Adds pre-trigger and trigger solutions.
  - Measures chemiluminescent emission to determine the quantity of myoglobin in the sample.
  - Aspirates contents of RV to liquid waste and unloads RV to solid waste.
  - Calculates the result.

Specimen Dilution Procedures

Specimens with a myoglobin value exceeding 1200.0 ng/mL will be flagged as ">1200.0" and may be diluted with the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol:

- If using the Automated Dilution Protocol, the system performs a 1:10 dilution of the specimen and automatically calculates the concentration of the specimen before dilution and reports the result.
- Specimens with a myoglobin value exceeding 12000.0 ng/mL are flagged with the code ">12000.0" when run using the Automated Dilution Protocol. These specimens may be diluted by following the Manual Dilution Procedure.

Manual Dilution Procedure:

- The suggested dilution for a myoglobin test is 1:20.
- Prior to diluting the specimen, dispense approximately 10 drops of ARCHITECT STAT Myoglobin Calibrator A into a clean test tube for use in the next step.
- Transfer 190 µL of ARCHITECT STAT Myoglobin Calibrator A from the test tube prepared in the prior step into another clean test tube and add 10 µL of the patient specimen.
- The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution. This will be the reported result. The dilution should be performed so that the diluted result reads greater than 40.0 ng/mL.

Calibration

- To perform an ARCHITECT STAT Myoglobin calibration, test the Calibrators A, B, C, D, E, and F in duplicate. A single sample of each ARCHITECT STAT Myoglobin Control level must be tested to evaluate the assay calibration. Ensure

that assay control values are within the concentration ranges specified in the control package insert. Calibrators should be priority loaded.

- Calibration Range: 0.0 – 1200.0 ng/mL.
- Once an ARCHITECT STAT Myoglobin calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
    - A reagent kit with a new lot number is used.
    - Controls are out of range.

Quality Control Procedures:

The recommended control requirement for the ARCHITECT STAT Myoglobin assay is that a single sample of each control level be tested once every 24 hours each day of use. If laboratory quality control procedures require more frequent use of controls to verify test results, follow those procedures.

The ARCHITECT STAT Myoglobin Control values must be within the acceptable ranges specified in the control package insert. If a control is out of its specified range, the associated test results are invalid and must be retested. Recalibration may be indicated.

Calculation

The ARCHITECT STAT Myoglobin assay uses a 4 Parameter Logistic Curve Fit (4PLC, Y-weighted) data reduction method to generate a calibration curve.

Expected Values:

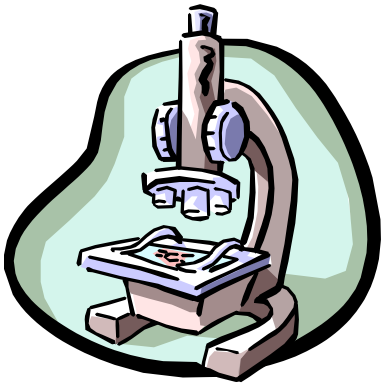
- Females: <106.0 ng/mL
- Males: <154.9 ng/mL

"The cut off levels and coefficients of variation (CV) of H-FABP, troponin-I, myoglobin and CK-MB used for the diagnosis of AMI in this study were >6.32ng/ml (at 99th percentile & CV = 7.94%) , >0.032 µg/L in males & >0.022 µg/L in females (at 99th percentile & CV = 10%), >106 ng/ml in males & >155 ng/ml in females (at 99th percentile & CV = 7.94%) and 24 IU/L respectively". These values were according to the recommendations of the reagent kit manufacturers.[72-77]

3.4 Statistical Analysis:

Continuous variables are presented as mean ± Standard deviation (SD) values and categorical data are presented as percent frequency of occurrence. Differences between means of two groups were assessed with the unpaired Student *t* test. Sensitivity, Specificity, Positive and Negative Predictive Values (PPV and NPV) were calculated and Receiver Operating Characteristic (ROC) curve analysis was done to assess the diagnostic validity for each marker at each time interval. For all the tests, p-value of 0.05 or less was considered for statistical significance. All the statistical procedures were performed using the Statistical Package for the Social Sciences statistical software (SPSS) version 16 for windows.

Chapter 5
Results



RESULTS

A total of 120 patients were included in this study. Of these 60 were acute myocardial infarction cases and 60 were non-cardiac chest pain otherwise healthy controls. Of 60 AMI cases 44 were males and 16 were females and of 60 controls 38 were males and 22 were females. The average age for the AMI cases was 60.7 years and that in controls was 54.82 years.

GRAPH – 1: GRAPHICAL REPRESENTATION OF DISTRIBUTION PATTERN OF STUDY GROUPS

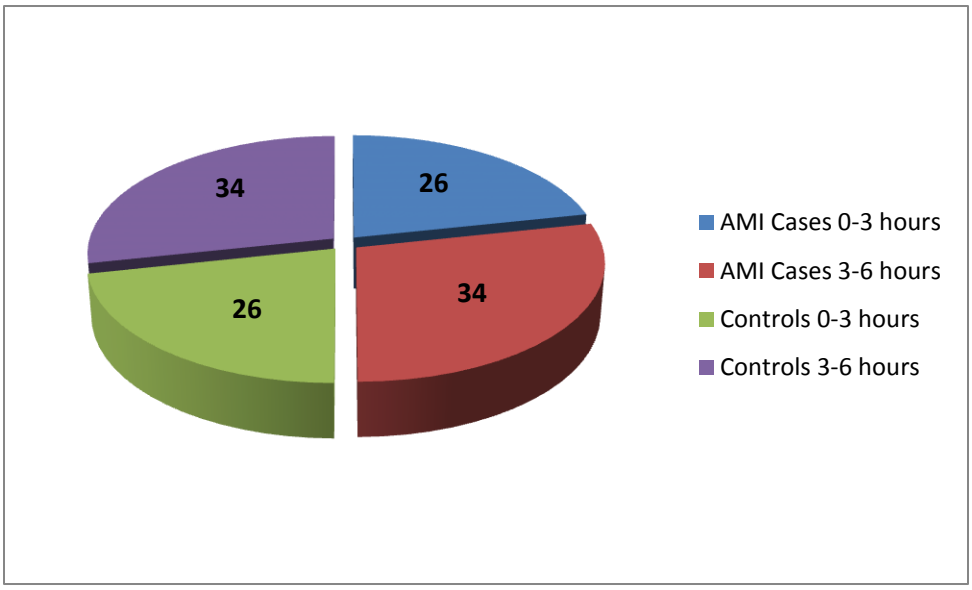


Figure – 12 shows the distribution pattern of study groups. The study included 60 AMI cases and equal number of age and sex matched non-AMI controls. In each group, 26 patients presented between 0 – 3 hours and 34 patients presented between 3 – 6 hours after the onset of chest pain.

TABLE – 7: AGEWISE DISTRIBUTION OF ACUTE MYOCARDIAL INFARCTION CASES AND CONTROLS

Age (Years)	Acute Myocardial infarction cases (0-3 hrs) (n = 26)	Acute Myocardial infarction cases (3-6 hrs) (n = 34)	Non – AMI Controls (0-3 hrs) (n = 26)	Non – AMI Controls (3-6 hrs) (n = 34)
≤ 30 years	--	--	02	03
31 – 40 years	02	01	02	02
41 – 50 years	01	08	07	06
51 – 60 years	10	10	07	12
61 – 70 years	08	10	06	09
71 – 80 years	02	02	02	02
≥ 81 years	03	03	--	--
Mean age ± SD	62.23 ± 12.75	60.27 ± 12.38	53.89 ± 13.93	53.79 ± 13.99

Table 7 shows agewise distribution of acute myocardial infarction (AMI) cases and controls studied. The study included 26 AMI cases presenting in 0 – 3 hrs after the onset of chest pain with a mean age (Mean age ± SD) of 62.23 ± 12.75 years and 34 AMI cases presenting between 3 – 6 hrs after the onset of chest pain with mean age of 60.27 ± 12.38 years. The mean age of 26 Non-AMI controls presenting in 0 – 3 hrs after the onset of chest pain was 53.89 ± 13.93 years and 34 Non AMI controls presenting between 3 – 6 hrs after the onset of chest pain was 53.79 ± 13.99 years. It is evident from the table that the incidence of AMI is higher in the age group of 40 – 60 years.

TABLE – 8: SEXWISE DISTRIBUTION OF ACUTE MYOCARDIAL INFARCTION CASES AND CONTROLS

Gender	Acute Myocardial infarction cases (0-3 hrs)	Acute Myocardial infarction cases (3-6 hrs)	Non-AMI Controls (0-3 hrs)	Non-AMI Controls (3-6 hrs)	Total
Males	20	24	18	20	82
Females	06	10	08	14	38
Total	26	34	26	34	120

Table – 8 shows the sex-wise distribution of acute myocardial infarction cases and controls in the study. Of 26 AMI cases presenting in 0 – 3 hrs after the onset of chest pain, 20 were males and 06 were females and among 34 AMI cases presenting between 3 – 6 hrs after the onset of chest pain 24 were males and 10 were females. 18 were males and 08 were females among 26 Non-AMI controls presenting 0 – 3 hrs after the onset of chest pain and among 34 Non AMI controls presenting between 3 – 6 hrs after the onset of chest pain 20 were males and 14 were females. It is evident from the table that the incidence of AMI is higher among males when compared to females.

TABLE – 9: BASELINE CHARACTERISTICS OF STUDY PARTICIPANTS

Clinical characteristic	AMI cases (n = 60)	Controls (n = 60)
Age (Years) (mean ± SD)	60.7 ± 12.2	54.82 ± 14.4
Males	44 (73.3%)	38 (63.3%)
Females	16 (26.7%)	22 (36.7%)
Smoking	18 (30%)	16 (26.7%)
Alcohol intake	16 (26.7%)	16 (26.7%)
Hypertension	25 (41.7%)	15 (25%)
Diabetes Mellitus	19 (31.7%)	15 (25%)
Hyperlipidemia	09 (15%)	05 (8.3%)
H/o previous IHD	03 (5%)	00
ECG changes • ST Elevation • T- wave inversion • Other changes (ST depression/ Tall T waves/ Q wave)	 51 (85%) 12 (20%) 05 (8.3%)	 -- 05 (8.3%) 03 (5%)

'Values are given as 'n' (%), unless otherwise specified; IHD = Ischemic Heart Disease'

The baseline clinical characteristics are depicted in table – 9. Patients diagnosed with AMI tended to be older than non-cardiac chest pain controls. AMI was more common among males when compared to females. The other demographics such as incidence of smoking, alcohol intake, hypertension, diabetes mellitus, hyperlipidemia, H/o previous IHD were more common in AMI cases when compared to controls. Among the ECG changes, ST elevation was seen among 85% of AMI cases, T wave insertion in 20% and other changes in 8.3% of the AMI cases. Interestingly, 13% of Non-AMI controls also presented with ECG changes suggestive of AMI.

TABLE – 10: SERUM MEAN LEVELS OF FOUR CARDIAC BIOMARKERS IN ACUTE MYOCARDIAL INFARCTION CASES AND CONTROLS

	AMI cases (n=60) (Mean ± SD)		**Controls (n=60) (Mean ± SD)**		***P*1 value**	***P*2 value**
	0 – 3 hrs (n = 26)	**3 – 6 hrs (n = 34)**	**0 – 3 hrs (n = 26)**	**3 – 6 hrs (n = 34)**		
H-FABP (ng/mL)	40.4 ± 56.3	76.3 ± 56.1	4.7 ± 7.3	6.7 ± 8.6	< 0.01	< 0.01
CK-MB (IU/L)	26.2 ± 19.2	36.9 ± 48.9	18.6 ± 9.5	25 ± 10.1	< 0.01	< 0.01
Troponin I (µg/L)	5.9 ± 16.2	8.5 ± 16.9	0.0048 ± 0.0077	0.0087 ± 0.0079	< 0.01	< 0.01
Myoglobin (ng/mL)	364.9 ± 363.9	584.5 ± 393.9	55.7 ± 53.4	90.8 ± 74.2	< 0.01	< 0.01

*P*1 = AMI cases (0-3 hr group) versus Controls (0-3 hr group)
*P*2 = AMI cases (3-6 hr group) versus Controls (3-6 hr group)

Table – 10 shows comparative analysis of the serum mean ± SD levels of four cardiac biomarkers measured in AMI cases and non-AMI controls at 0-3 hrs and 3-6 hrs after the onset of chest pain. It is evident from the above table that, The serum mean levels of CK-MB, troponin I, myoglobin and H-FABP were significantly higher in AMI cases when compared to that of non-AMI controls in both 0 – 3 hrs and 3 – 6 hrs groups. The difference in the mean levels of all the four cardiac biomarkers between cases and controls was statistically significant in both the time intervals ($p < 0.01$).

TABLE – 11: DIAGNOSTIC PERFORMANCES OF FOUR CARDIAC BIOMARKERS FOR THE EARLY DIAGNOSIS OF AMI WITHIN 3 HOURS AND 3 -6 HOURS AFTER THE ONSET OF CHEST PAIN

	Sensitivity (%)		Specificity (%)		PPV (%)		NPV (%)	
	0 – 3 hrs	3 – 6 hrs	0 – 3 hrs	3 – 6 hrs	0 – 3 hrs	3 – 6 hrs	0 – 3 hrs	3 – 6 hrs
H-FABP (ng/mL)	92.3	94.1	88.5	79.4	88.9	82.1	92.0	93.1
CK-MB (IU/L)	23.1	52.9	61.5	58.8	37.5	56.3	44.5	55.6
Troponin I (µg/L)	96.2	100	100	100	100	100	96.3	100
Myoglobin (ng/mL)	73.1	88.2	76.9	70.6	76.0	75.0	74.1	85.7

PPV = Positive Predictive Value; NPV = Negative Predictive value

Table – 11 shows the diagnostic performance characteristics in the form of sensitivity, specificity, positive and negative predictive values of all the four cardiac biomarkers for the diagnosis of acute myocardial infarction at 0-3 hrs and 3-6 hrs after the onset of chest pain. It is evident from the table that, the sensitivity, specificity, positive and negative predictive values of H-FABP were significantly higher than that of CK-MB and myoglobin and slightly lesser than that of troponin I in both 0-3 hrs and 3-6 hrs groups.

GRAPH – 2: ROC CURVE ANALYSIS OF H-FABP, CK-MB, TROPONIN I AND MYOGLOBIN IN 0 - 3 HOURS GROUP

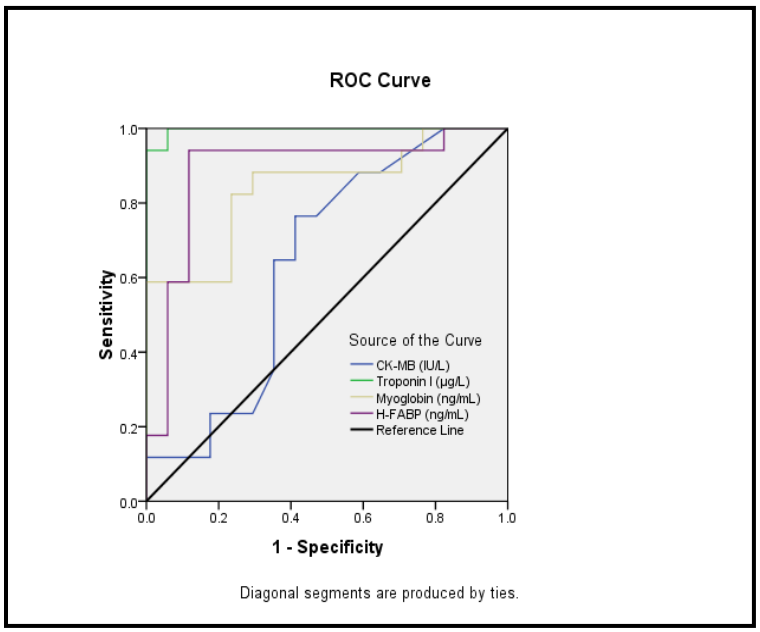


Table – 12: Area under ROC curve for cardiac markers in 0 – 3 hours group

	AUC	*'P'* value	Confidence Interval	
			Lower limit	Upper limit
H-FABP	0.886	0.000	0.756	1.015
CK-MB	0.640	0.163	0.446	0.834
Troponin I	0.997	0.000	0.985	1.008
Myoglobin	0.841	0.001	0.705	0.977

AUC = Area under curve

Figure – 13 and Table – 12 shows the Receiver Operating Characteristic (ROC) curve analysis of CK-MB, troponin I, myoglobin and H-FABP for their diagnostic accuracy at 0-3 hrs after the onset of chest pain. It is evident from the table that, troponin I (AUC = 0.997) had highest area under curve (AUC) and hence highest diagnostic accuracy followed by H-FABP (AUC = 0.886), myoglobin (AUC = 0.841) and CK-MB

(AUC = 0.64) within 3 hours after the onset of chest pain for the diagnosis of acute myocardial infarction.

GRAPH – 3: ROC CURVE ANALYSIS OF H-FABP, CK-MB, TROPONIN I AND MYOGLOBIN IN 3 – 6 HOURS GROUP

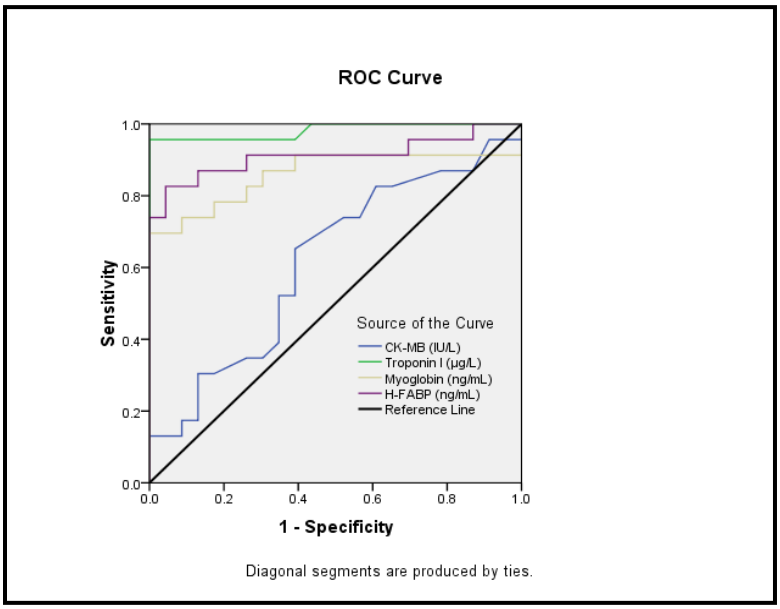


Table – 13: Area under ROC curve for cardiac markers in 3 – 6 hour group

	AUC	*'P'* value	Confidence Interval	
			Lower limit	Upper limit
H-FABP	0.911	0.000	0.816	1.007
CK-MB	0.616	0.177	0.452	0.781
Troponin I	0.982	0.000	0.946	1.019
Myoglobin	0.860	0.000	0.738	0.983

AUC = Area under curve

Figure – 14 and Table – 13 shows the Receiver Operating Characteristic (ROC) curve analysis of CK-MB, troponin I, myoglobin and H-FABP for their diagnostic accuracy at 3-6 hrs after the onset of chest pain. It is evident from the table that, troponin I had highest area under curve (AUC) and hence highest diagnostic accuracy (AUC =

0.982) followed by H-FABP (AUC = 0.911), myoglobin (AUC = 0.860) and CK-MB (AUC = 0.616) between 3 - 6 hours after the onset of chest pain for the diagnosis of acute myocardial infarction.

Chapter 6
Discussion

DISCUSSION

The evaluation of acute chest pain has traditionally been a challenge. The early and accurate diagnosis of acute coronary syndrome (ACS) is critically important, because delay in the diagnosis and treatment can lead to serious complications and even death apart from increasing the economic burden.[73] Ischemic heart disease (IHD) is the leading cause of death among adults and represents a spectrum from chronic stable angina to acute myocardial ischemia (AMI). It is responsible for more than 500,000 deaths annually. 'AMI is a common cause of sudden and unexpected death'. Early diagnosis of AMI is crucial, as this allows earlier initiation of appropriate treatment and improves patient outcome.[74]

The diagnosis of AMI requires a "clinical picture suggestive of myocardial ischemia, including ischemic symptoms, characteristic ECG changes, new Q waves on ECG, new loss of myocardium or new wall motion abnormality on echocardiography, coexistent with a rise and fall in serum levels of cardiac biomarkers indicative of myocardial necrosis with at least 1 value above the 99th percentile of the upper limit of normal". However, the symptoms are often atypical or absent, and around 33% of the patients arriving at the emergency department with AMI may not have chest pains.[78] Although ECG remains the best test to detect AMI in the emergency department, it has relatively low sensitivity. Generally, ST segment elevations in a standard 12-lead ECG suggest transmural injury, while ST segment depressions suggest subendocardial ischaemia. However, in the initial ECG, 50% of patients will not have diagnostic ST segment elevations. Cardiac markers are also useful tools in the diagnosis of AMI.[79] In the beginning, the biochemical markers of myocardial damage used were 'lactate

dehydrogenase (LDH) and its isoenzymes, and aspartate aminotransferase (AST)', but their use was limited, because of their poor cardiac specificity. Later on, with the invention creatine kinase-MB (CK-MB), Specificity improved, because it is abundant in the heart but still is found in other tissues. Presently, cardiac troponins (cTn) are the most specific and gold standard biomarker for MI, however, its appearance in the serum is delayed (6 hours following AMI) and also it lacks the ability to detect ischemia in the absence of necrosis. Myoglobin is an early marker (within 1-2 hrs) of myocardial ischemia but less specific. Hence, the search for an ideal cardiac marker is still on. 'Heart-type fatty acid binding protein' is one of the most promising novel markers of AMI.[75]

There have been considerable advances over the years that have led to a comprehensive understanding of the 'pathophysiology and bimolecular basis of coronary artery disease (CAD)'. As a consequence, novel cardiac biomarkers are an exciting and fascinating area of research. Novel technologies have allowed us to screen large samples of blood in a much reduced time than ever before. This provides a larger scope for researchers to apply their understanding according to the ever-growing clinical demand. There has been rapid growth in the number of novel biomarkers that emphasizes the importance of their evaluation. An ideal biomarker should convincingly demonstrate its value and utility in helping with diagnosis, disease stratification and prognostication, beyond that of the existing markers.[76] Good clinical decision making requires a thorough understanding of the strengths and limitations of each diagnostic test. The test characteristics convey information about the 'performance of a test' which are expressed in terms of 'sensitivity, specificity, positive and negative predictive values'.[77]

In the present study, the incidence of myocardial infarction was more common in males, smokers, and those having hypertension, diabetes and hyperlipidemia, which are the known risk factors for coronary heart disease.[78] In cases diagnosed with AMI, only 83.5% of the patients were having ECG changes and among the control group, 8.3% of the patients with non-cardiac chest pain also presented with ECG changes suggestive of AMI [Table – 9]. Interestingly, clinicians use the ECG, a test with considerably less sensitivity and specificity, in their daily chest pain decision-making. In a multicentric chest pain study, Rouan et al. noted retrospectively that 10% of Ml patients presented with completely normal or non-specific ECGs, and only 79% presented with new ischaemic findings.[79] Similarly, Young and Green reported in a prospective study that only 56% of adult Ml patients presented with new ST-segment, T-wave, or Q-wave ECG findings. Furthermore, physicians may misinterpret ECGs in the emergency department.[80]

Admission ECG has certain limitations for the diagnosis of AMI and cannot be interpreted in the following conditions; i) Conduction defects Eg. 'left bundle branch block (LBBB)' ii) Prior presence of 'Q waves and ST-T wave changes', as in cases of old infarcts and digoxin therapy, respectively; iii) Severe left ventricular hypertrophy with ST-T wave changes; iv) 'Posterior infarct or right ventricular infarct', in which no characteristic ECG changes of MI are seen.[7]

In our study, mean serum concentrations of H-FABP, troponin I, myoglobin and CK-MB activity were significantly higher ($p< 0.01$) in AMI cases when compared to controls both in 0-3 hrs and 3-6 hrs groups. Also, the mean levels of cardiac markers were much higher in 3-6 hrs group when compared to 0-3 hrs group of AMI cases [Table – 10]. This finding is in agreement with the studies conducted earlier by Elmadbouh I et

al.[5], Glatz JFC et al.[35], and Pasaoglu H et al.[47]. However, Orak et al.[51] found that there was a significant difference in the mean levels of H-FABP and CK-MB in ACS cases when compared to the controls (p = 0.000), whereas no difference was observed for troponin I (p = 0.013) within 6 hrs.

In our study, H-FABP was found to have greater diagnostic performance in the form of sensitivity, specificity, PPV and NPV than CK-MB and myoglobin in patients with suspected AMI both within 3 hrs and 3–6 hrs after the onset of chest pain. The diagnostic performance characteristics of H-FABP were comparable to troponin I but never superior both within 3 hrs and 3–6 hrs after the onset of chest pain [Table – 10]. Several studies have compared the diagnostic value of H-FABP with CK-MB, troponin I and myoglobin in patients with chest pain and arrived at conflicting results. Elmadbouh et al.[5], Pasaoglu et al.[47], Alhadi et al.[50], Orak et al.[51], and McMahon et al.[54] have found that, H-FABP as more sensitive and specific cardiac marker than CK-MB, troponin I and myoglobin within 6 hrs of onset of chest pain. In contrast, Alansari and Croal[40] suggested that H-FABP and myoglobin provide little clinical value compared to troponin I, when measured at presentation in patients presenting with chest pain (3–12 hrs). In a multicentric study conducted by Freund et al.[55], H-FABP had no additional value over cardiac troponin I for the diagnosis of myocardial necrosis (STEMI and NSTEMI) in ED patients with chest pain of less than 6 hrs duration.

The sensitivity and specificity vary across the different threshold / cut off values and the sensitivity is inversely related with specificity. Hence, the plot of sensitivity versus 1-specificity called ‘receiver operating characteristic (ROC) curve’ and the ‘area under the curve (AUC)’, an effective measure of accuracy has been considered with a

meaningful interpretations. This curve plays a central role in evaluating diagnostic ability of tests to discriminate the true state of subjects, finding the optimal cut off values, and comparing two alternative diagnostic tests when each test is performed on the same subject.[81]

Hence, we examined the diagnostic ability of each cardiac marker for the diagnosis of AMI at 0–3 hrs and 3–6 hrs after the onset of chest pain by ROC curve analysis [Fig – 13 & 14; Table – 12 & 13]. In both 0–3 hrs and 3–6 hrs groups, the area under ROC curve and diagnostic ability of H-FABP were much better than CK-MB and myoglobin but they were lesser in comparison with troponin I. The AUC values of these markers were statistically significant in both the groups ($p < 0.001$) except for CK-MB ($p = 0.163$ in 0-3 hrs group; $p = 0.177$ in 3-6 hrs group). This finding is in full agreement with the studies conducted by Kim et al.[53] and Freund et al.[55]. However Orak et al.[51] and McMahon et al.[54] showed that, AUC for H-FABP was highest of all markers at 0–6 hrs after chest pain followed by CK-MB, troponin I and myoglobin.

Superior diagnostic performance of H-FABP over CK-MB and myoglobin for the early diagnosis of AMI may be because of the following reasons: (1) H-FABP has small molecular weight (15 kDa) when compared to CK-MB (80 kDa) and myoglobin (18 kDa). A small sized marker will help in early identification of myocardial damage, because it is more rapidly released into blood; (2) Relative specificity of H-FABP for the cardiac tissue. Its concentration within the myocardial tissue is 10 times higher when compared to that of skeletal muscle. However, CK-MB and myoglobin are less specific for cardiac tissue;[7] CK-MB forms 15 – 40% of total CK activity in the cardiac muscle, and the remainder is CK-MM. Trace amounts of CK-MB are found in skeletal muscle (2

– 3% of the total CK activity).[16] Concentration of myoglobin is approximately twofold lower in cardiac than in skeletal muscle. (3) H-FABP appears in the plasma early (within 2 hrs) with a peak at 6 hrs after the cardiac damage. However CK-MB begins to increase in the blood between 4 to 6 hrs after the onset of infarction and peaking at 16–20 hrs. (4) Normal plasma baseline H-FABP concentration (< 5 µg/L) is several folds lower than that of CK-MB and myoglobin. Thus 'early detection of AMI' is possible if there is even minimal increase in the concentration of the marker in the plasma.[7]

To summarize the findings of our study, the diagnostic performance of H-FABP in the early detection of AMI was clearly superior to CK-MB and myoglobin but little inferior to troponin I, but the results of previous studies are conflicting. Although, cardiac troponins are most widely used for the diagnosis of AMI, because of delayed rise in the blood and its inability to detect early phase ischemia in the absence of necrosis, many AMI patients and those who are at risk of cardiac adverse outcome remain undetected with troponin assay alone. Also, Novel biomarkers such as H-FABP can provide information in addition to those already provided by troponin assay, which include further stratification of patients, early detection of myocardial ischemia, and determine patients' prognosis.[82] Currently, as there is no single ideal cardiac marker, it is recommended that two biochemical markers should be used for routine AMI diagnosis; "an early marker (reliably increased in blood within 6 hrs of the onset of symptoms) and a definitive marker (increased in blood after 6-9 hrs, with high sensitivity and specificity for myocardial injury, and remaining abnormal for several days after onset)".[83]

Troponin I assay when combined with H-FABP, improves the sensitivity, specificity and NPV for AMI detection within 6 hrs of chest pain. Pasaoglu et al.[47] found

the combined measurement of 'H-FABP and troponin I' had 98%, 99% and 100% NPV, at 3 to 6 hrs, 6 to 12 hrs and > 12 hrs after the onset of chest pain, respectively. Also, this combination had highest area under ROC curve (0.915) at 3-6 hrs when compared to that of measurement of H-FABP (0.894) or trponin I (0.851) alone. These outcomes demonstrate that this blend of markers can be utilized successfully as 'rule out test' when in doubt, to distinguish those not having AMI at an early time period of < 6 hrs after the onset of chest pain. McCann et al.[68] additionally found that, this combined measurement of markers has improved sensitivity in chest pain patients within 4 – 12 hrs of onset, for AMI diagnosis. Also, Li et al.[84] reported a 20% increase in sensitivity for the this combination of markers, compared to the use of troponin I alone, and also they concluded that, troponin T and H-FABP is currently the 'most effective combination for the diagnosis of early AMI'.

When considered individually, H-FABP also has certain advantages over troponin I which include, H-FABP can be used for detection of 're-infarction - a well recognized complication following AMI', which has worse prognosis. H-FABP returns to the baseline concentrations about 20 hrs after the symptom onset, where as 'CK-MB and troponins' take several days. And newly developed immune-turbidimetric assay for the measurement of H-FABP concentration requires just 10 minutes of time as against Chemiluminescence assay for troponins which requires 90 – 120 minutes.[9] Shorter assay time of H-FABP can be very helpful for the early diagnosis / rule out of AMI.

A few studies[5,47,50,51,54] indicated that the estimation of serum H-FABP has greater diagnostic value than troponin I, especially within 3 hrs after the beginning of manifestations of AMI. Some other studies[40, 55] demonstrated that, H-FABP does not give

extra helpful information to troponin I. Reasons for the conflicting results can be "the duration of MI at the time of sampling, different study settings (cardiology units, Emergency department or pre-hospital etc.), different methods and cut off values for the H-FABP used".

It has been proposed that, the serum H-FABP level can be high in renal disorders. H-FABP, being a low molecular weight protein, is largely excreted through kidneys. Renal excretion is reduced in patients with disorders of the kidney. But, cardiac troponins also exhibit this limitation. H-FABP concentration in the blood can also be raised in severe skeletal muscle damage, because it is expressed in skeletal muscle, although in quantities several folds lesser than heart. However, this is also seen with cTn. It has been reported that up to 30% of patients presenting with raised cTn do not have typical ACS.[7]

Limitations: Our study presents few limitations. First, sample size is relatively small but it fulfils the statistical norms. Second, we could not do the serial measurements of the cardiac markers for the assessment of their kinetics.

Chapter 7
Conclusions

CONCLUSION

The diagnostic ability of H-FABP is greater than CK-MB and myoglobin but slightly lesser than troponin I for the early diagnosis of acute myocardial infarction within 6 hours of onset of chest pain, as demonstrated by the sensitivity, specificity, positive predictive value, negative predictive value and receiver operating characteristic curves. H-FABP can be used as an additional diagnostic tool for the early diagnosis of myocardial infarction along with troponin I.

Chapter 8
Summary

SUMMARY

Early diagnosis and therapeutic intervention can improve the outcome of acute myocardial infarction (AMI). However, there are no satisfactory cardiac biomarkers for the diagnosis of AMI within 6 hours of onset of symptoms. Among novel biochemical markers of AMI, heart-type fatty acid binding protein (H-FABP) is of particular interest.

The study aimed to investigate whether H-FABP measurement provides additional diagnostic value to that of conventional cardiac markers in AMI within first 6 hours after the onset of symptoms. The objectives of the study were to compare the sensitivity, specificity, positive predictive value, negative predictive values and also the diagnostic ability of H-FABP with the conventional cardiac biomarkers (CK-MB, Troponin I and Myoglobin) in suspected patients of acute myocardial infarction presenting with chest pain within 3 hrs and 3 – 6 hrs after the onset of chest pain,

A total of 120 patients presenting with acute chest pain within 6 hours of onset, suggestive of AMI were included in the present study according to the inclusion and exclusion criteria. In all the cases and controls, complete clinical history, ECG, echocardiography and other routine investigation findings were noted. Serum H-FABP concentration was measured by immunoturbidimetric method, serum troponin I and myoglobin concentrations were measured by chemiluminescence immunoassay and serum CK-MB activity was estimated by immuno-inhibition method. Diagnosis of AMI was done according to the European Society of Cardiology/ American College of Cardiology Committee (ESC/ACCC) Criteria. 60 cases were diagnosed as having AMI cases and remaining 60 cases were age and sex matched non-cardiac chest pain controls.

The cases and controls were further divided into 2 subgroups depending on the time since onset of chest pain as those subjects within 3 hours and those between 3-6 hours of onset of chest pain. Data was presented as mean ± SD values. Differences between means of two groups were assessed by Student t-test. Sensitivity, Specificity, Positive predictive value, Negative predictive values were calculated and ROC curve analysis was done to assess the diagnostic validity of each study parameter.

We found that, the serum mean levels of H-FABP, CK-MB, troponin I, and myoglobin were significantly higher ($p > 0.01$) in AMI cases when compared to that of non-AMI controls in both 0 – 3 hrs (40.4 ± 56.3, 26.2 ± 19.2, 5.9 ± 16.2, 364.9 ± 363.9 vs 4.7 ± 7.3, 18.6 ± 9.5, 0.0048 ± 0.0077, 55.7 ± 53.4 respectively) and 3 – 6 hrs (76.3 ± 56.1, 36.9 ± 48.9, 8.5 ± 16.9, 584.5 ± 393.9 vs 6.7 ± 8.6, 25 ± 10.1, 0.0087 ± 0.0079, 90.8 ± 74.2 respectively) groups. The sensitivity, specificity, positive and negative predictive values of H-FABP (92.3%, 88.5%, 88.9%, 92.0% in 0-3 hr group; 94.1%, 79.4%, 82.1%, 93.1% in 3-6 hr group, respectively) were significantly greater than CK-MB (23.1%, 61.5%, 37.5%, 44.5% in 0-3 hr group; 52.9%, 58.8%, 56.3%, 55.6% in 3-6 hrs group, respectively) and myoglobin (73.1%, 76.9%, 76.0%, 74.1% in 0-3 hrs group; 88.2%, 70.6%, 75.0%, 85.7% in 3-6 hrs group, respectively) but were lesser than Troponin I (96.2%, 100%, 100%, 96.3% in 0-3 hrs group; 100%, 100%, 100%, 100% in 3-6 hrs group, respectively) in patients with suspected AMI in both within 3 hours and 3 – 6 hours groups. Receiver Operating Characteristic (ROC) curves demonstrated greatest diagnostic ability for Troponin I (AUC = 0.997 & P = 0.000 in 0-3 hr group; AUC = 0.982 & P = 0.000 in 3-6 hrs group) followed by H-FABP (AUC = 0.886 & P = 0.000 in 0-3 hrs group; AUC = 0.911 & P = 0.000 in 3-6 hrs group), myoglobin (AUC = 0.841 &

P = 0.001 in 0-3 hrs group; AUC = 0.860 & P = 0.000 in 3-6 hrs group) and CK-MB (AUC = 0.640 & P = 0.163 in 0-3 hrs group; AUC = 0.616 & P = 0.177 in 3-6 hrs group) within 3 hrs and 3 – 6 hrs after the onset of chest pain.

To summarize the findings of our study, the diagnostic performance of H-FABP in the early detection of AMI was clearly superior to CK-MB and myoglobin but little inferior to troponin I, but the results of previous studies are conflicting. Although, cardiac troponins are most widely used for the diagnosis of AMI, because of delayed rise in the blood and its inability to detect early phase ischemia in the absence of necrosis, many AMI patients and those who are at risk of cardiac adverse outcome remain undetected with troponin assay alone. Novel biomarkers such as H-FABP can provide information in addition to those already provided by troponin assay, which include further stratification of patients, early detection of myocardial ischemia, and determine patients' prognosis. Also, H-FABP can be used for detection of 're-infarction - a well recognized complication following AMI. H-FABP returns to the baseline concentrations about 20 hrs after the symptom onset, where as troponins take several days. And newly developed immune-turbidimetric assay for the measurement of H-FABP concentration requires just 10 minutes of time as against Chemiluminescence assay for troponins which requires 90 – 120 minutes. Shorter assay time of H-FABP can be very helpful for the early diagnosis / rule out of AMI. Both cardiac troponins and H-FABP values should be cautiously interpreted in the presence of renal and skeletal muscle damage.

Currently, as there is no single ideal cardiac marker, it is recommended that two biochemical markers should be used for routine AMI diagnosis; "an early marker (reliably increased in blood within 6 hrs of the onset of symptoms) and a definitive

marker (increased in blood after 6-9 hrs, with high sensitivity and specificity for myocardial injury, and remaining abnormal for several days after onset)". Thus, Troponin I assay when combined with H-FABP, can improve the sensitivity, specificity and NPV for AMI detection within 6 hrs of chest pain.

It can be concluded that, the diagnostic ability of H-FABP is greater than CK-MB and myoglobin but slightly lesser than troponin I for the early diagnosis of acute myocardial infarction within 6 hours of onset of chest pain, as demonstrated by the sensitivity, specificity, positive predictive value, negative predictive value and receiver operating characteristic curves. H-FABP can be used as an additional diagnostic tool for the early diagnosis of myocardial infarction along with troponin I.

Chapter 9
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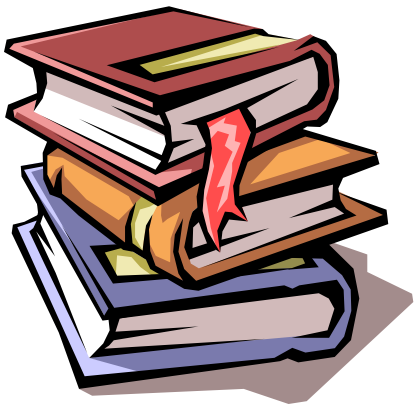

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Chapter 10
Annexures

ANNEXURE – I

CASE REPORT FORM

DEPARTMENT OF BIOCHEMISTRY,
BLDE (DEEMED TO BE UNIVERSITY) SHRI B M PATIL MEDICAL COLLEGE, VIJAYAPUR – 590001, KARNATAKA.

TITLE OF THE STUDY: Heart-Type Fatty-Acid Binding Protein, in Early Detection of Acute Myocardial Infarction – Comparison with CK – MB, Troponin I and Myoglobin.

Name of the Ph.D student: Dr Anand

Name of the Guide: Dr B B Devaranavadagi

1. Case / Control No.:
2. Name:
3. Age:
4. Sex:
5. Address:
6. Occupation:
7. IP No.:
8. Date of admission:
9. Date and time of collection of blood sample:
10. **History of presenting complaints:**

11. **Past History:** H/O similar complaints in the past – Yes / No

    H/O Jaundice / Renal disease / Skeletal muscle disease / Recent coronary artery disease / alcohol intake / diabetes mellitus / in the past.

12. **Family History:** H/O similar complaints in the family members – Yes / No

13. Personal History:

i. Diet – Veg. / Mixed

ii. Appetite – Normal / Decreased

iii. Sleep – Normal / Disturbed

iv. Bowel and bladder habits – Regular / Disturbed

v. Alcohol consumption – Yes / No; if yes, then

Duration - Amount - Frequency -

vi. Smoking – Yes / No; if yes, then

Duration - Frequency –

14. Treatment History:

15. General Physical Examination:

Built	Well /Average / Poor
Nourishment	Well / Average / Poor
Pallor	present/absent
Icterus	present/absent
Clubbing	present/absent
Lymphadenopathy	present/absent
Oedema	present/absent

Vitals:

PR:	BP:
RR:	Temp:
Weight:	

16. Systemic Examination:

i. Cardiovascular System

ii. **Respiratory system**

iii. **Central Nervous System**

iv. **Per abdomen examination**

17. Provisional Diagnosis:

18. Investigations

1. Electrocardiogram findings:

2. Echocardiography findings:

3. Routine Investigations:

4. Serum CK-MB:
5. Serum Troponin I:
6. Serum Myoglobin:
7. Serum H-FABP:

19. Final Diagnosis:

ANNEXURE - II

INFORMED CONSENT AND PATIENT INFORMATION FORM

Department of Biochemistry, BLDE (Deemed to be University) Shri B M Patil Medical College, Hospital & Research Centre, Vijayapur – 586103, Karnataka

Patient Name:

Age:

Sex:

Address:

I _____ Relation to patient _____ hereby confirm that, I have been informed in detail about the procedures involved the "HEART-TYPE FATTY ACID-BINDING PROTEIN, IN EARLY DETECTION OF ACUTE MYOCARDIAL INFARCTION – COMPARISON WITH CK – MB, TROPONIN I AND MYOGLOBIN" in my own language. I have also understood the advantages and any disadvantages involved in the same. I was given an opportunity to ask questions and all of them have been answered to my satisfaction. I therefore give my consent for procedures involved in the above mentioned study to be performed on the patient.

Name & Signature of the Patient/ Parent / Guardian

Date and Time:

Name & Signature of Doctor in charge

ಅನುಮೋದನೆ ಒಪ್ಪಿಗೆ ಪತ್ರ

ಜೀವರಸಾಯನ ಶಾಸ್ತ್ರ ವಿಭಾಗ, ಶ್ರೀ ಬಿ.ಎಂ. ಪಾಟೀಲ್ ವೈದ್ಯಕೀಯ ಕಾಲೇಜು ಮತ್ತು ಆಸ್ಪತ್ರೆ,

ವಿಜಯಪುರ - ೫೮೬೧೦೩. ಕರ್ನಾಟಕ ರಾಜ್ಯ

ರೋಗಿಯ ಹೆಸರು :

ವಯಸ್ಸು :

ಲಿಂಗ :

ವಿಳಾಸ :

ನಾನು ..... ಧೃಢೀಕರಿಸುವುದೇನೆಂದರೆ, ನಮಗೆ ''ಹೃದಯಾಘಾತದ ಶೀಘ್ರ ಪತ್ತೆ ಹಚ್ಚುವಿಕೆಯಲ್ಲಿ ಹಾರ್ಟ-ಟೈಪ್ ಫ್ಯಾಟಿ ಎಸಿಡ್ ಬೈಂಡಿಂಗ್ ಪ್ರೋಟಿನ್- ಸಿಕೆ-ಎಂಬಿ, ಟ್ರೋಪೊನಿನ್, ಮಯೋಗ್ಲೊಬಿನ್‌ಗಳ ಜೊತೆ ಹೋಲಿಕ"ೆ ಎಂಬ ಸಂಶೋಧನೆಗಾಗಿ ನಮ್ಮ ವೈದ್ಯಕೀಯ ಪರೀಕ್ಷೆ ಮತ್ತು ರಕ್ತದ ಮಾದರಿಯನ್ನು ಪರೀಕ್ಷೆಗಾಗಿ ಸಂಗ್ರಹಿಸುವ ಉಪಯೋಗದ ಬಗ್ಗೆ ತಿಳಿದಿರುತ್ತದೆ ಮತ್ತು ಇದರ ಉಪಯೋಗದ ಪರಿಣಾಮಗಳನ್ನು ವೈದ್ಯರು ತಿಳಿಸಿರುತ್ತಾರೆ. ಈ ವೈದ್ಯಕೀಯ ಪರೀಕ್ಷೆಯ ಬಗ್ಗೆ ಇರುವ ಎಲ್ಲಾ ನನ್ನ ಸಂಶಯಗಳನ್ನು ವೈದ್ಯರು ವಿವರಿಸಿರುತ್ತಾರೆ. ನಾನು ಇದರ ಲಾಭ ಮತ್ತು ಹಾನಿಕಾರಿಕಗಳನ್ನು ತಿಳಿದಿರುತ್ತೇನೆ. ಆದ್ದರಿಂದ ನಾನು ನನ್ನ ಸ್ವಇಚ್ಛೆಯಿಂದ ನನ್ನನ್ನು ಈ ವೈದ್ಯಕೀಯ ಪರೀಕ್ಷೆಗೆ ಒಳಪಡಿಸುತ್ತೇನೆ.

ರೋಗಿ/ಪೋಷಕರ ಸಹಿ:

ದಿನಾಂಕ ಮತ್ತು ಸಮಯ :

ತಪಾಸಣಾ ವೈದ್ಯರ ಸಹಿ:

ಸಾಕ್ಷಿಗಳ ಸಹಿ: ೧)

೨)

PATIENT INFORMATION FORM

I Dr. ANAND, Ph.D student in the Department of Biochemistry, BLDE (Deemed to be University) Shri B M Patil Medical College, Hospital & Research Centre, Vijayapur, will be conducting a "HEART-TYPE FATTY-ACID BINDING PROTEIN, IN EARLY DETECTION OF ACUTE MYOCARDIAL INFARCTION – COMPARISON WITH CK – MB, TROPONIN I AND MYOGLOBIN". You will be given complete information about the research and you will be invited to be a part of the study. I assure you that the details obtained during the course of study will be kept confidential and will not be revealed to anyone. You are free to decide anytime whether you want to participate in the study or not after going through the information given to you. The research procedure will involve asking for the complete history including the personal information and history of presenting illness, physical examination and collection of the blood sample. This as such will have no adverse effects on you. Wherever you will have queries I will be happy to explain. You will not be compensated by any means for participating in the study. If you are not interested to participate in the study or if you feel uncomfortable, you can withdraw or can refuse to participate in the study at any time.

Name and address of the principal investigator:

Dr. ANAND,
Ph.D student,
Department of Biochemistry,
BLDE (Deemed to be University)
Shri B M Patil Medical College, Hospital & Research Centre,
Vijayapur – 590001. Karnataka
Mobile No.: 7259678077.

ರೋಗಿಗೆ ತಿಳುವಳಿಕೆ ನೀಡುವ ಪತ್ರ

ನಾನು ಡಾ॥ ಆನಂದ್, ಜೀವರಸಾಯನ ಶಾಸ್ತ್ರ ವಿಭಾಗ, ಶ್ರೀ ಬಿ.ಎಂ. ಪಾಟೀಲ್ ವೈದ್ಯಕೀಯ ಕಾಲೇಜು ಮತ್ತು ಆಸ್ಪತ್ರೆ,ವಿಜಯಪುರ ದಲ್ಲಿ ಪಿ.ಎಚ್.ಡಿ ಪದವಿ ವಿದ್ಯಾರ್ಥಿಯಾಗಿರುತ್ತೇನೆ.

ನಾನು ''ಹೃದಯಾಘಾತದ ಶೀಘ್ರ ಪತ್ತೆ ಹಚ್ಚುವಿಕೆಯಲ್ಲಿ ಹಾರ್ಟ-ಟೈಪ್ ಫ್ಯಾಟಿ ಎಸಿಡ್ ಬೈಂಡಿಂಗ್ ಪ್ರೋಟಿನ್-ಸಿಕೆ-ಎಂಬಿ, ಟ್ರೋಪೊನಿನ್, ಮಯೋಗ್ಲೊಬಿನ್‌ಗಳ ಜೊತೆ ಹೋಲಿಕೆ'' ಎಂಬ ಸಂಶೋಧನೆಯನ್ನು ನಡೆಸುತ್ತಿದ್ದೇನೆ. ನಾನು ಈ ಸಂಶೋಧನೆಯ ಬಗ್ಗೆ ನಿಮಗೆ ಪೂರ್ಣ ಪ್ರಮಾಣದಲ್ಲಿ ತಿಳಿಸುವೆ. ಈ ವಿವರಣೆ ನಂತರ ನಿಮ್ಮ ಒಪ್ಪಿಗೆ ಪಡೆದು ಈ ಸಂಶೋಧನೆಗೆ ಒಳಪಡಿಸುತ್ತೇನೆ. ನೀವು ನೀಡಿದ ವಿವರಣೆಯನ್ನು ಎಲ್ಲೂ ಕೂಡ ಬಹಿರಂಗ ಪಡಿಸುವುದಿಲ್ಲವೆಂದು ನಾನು ನಿಮಗೆ ವಿಶ್ವಾಸ ನೀಡುತ್ತೇನೆ. ತಾವೂ ಯಾವ ಕ್ಷಣದಲ್ಲಿಯೂ ಈ ಸಂಶೋಧನೆಯಿಂದ ಹೊರ ಹೋಗಲು ಇಚ್ಛಿಸಿದಲ್ಲಿ ತಮಗೆ ಸಂಪೂರ್ಣ **ಸ್ವಾ**ತಂತ್ರ್ಯ ನೀಡಲಾಗುತ್ತದೆ. ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ನಿಮ್ಮ ಎದೆ ನೋವಿನ ಬಗೆಗಿನ ಸಂಪೂರ್ಣ ಮಾಹಿತಿಯನ್ನು ಕೇಳಿ ಪಡೆಯಲಾಗುವುದು ಮತ್ತು ವೈದ್ಯಕೀಯ ಪರೀಕ್ಷೆ ನಡೆಸಲಾಗುವುದು ನಂತರ ರಕ್ತದ ಮಾದರಿಯನ್ನು ಪರೀಕ್ಷೆಗಾಗಿ ಸಂಗ್ರಹಿಸಲಾಗುವದು. ಇದರಿಂದ ನಿಮಗೆ ಯಾವುದೇ ಹಾನಿ ಆಗುವುದಿಲ್ಲ. ಈ ಮಾಹಿತಿಗಳನ್ನು ತಿಳಿದ ನಂತರ ನಿಮಗಿಷ್ಟ ಬಂದವರೊಂದಿಗೆ ಚರ್ಚಿಸಿ ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗಿಯಾಗುವ ನಿರ್ಣಯ ತೆಗೆದುಕೊಳ್ಳಲು ನೀವು ಸಂಪೂರ್ಣ ಸ್ವತಂತ್ರರು. ಈ ಸಂಶೋಧನೆಯ ಪ್ರಶ್ನಾವಳಿಯಲ್ಲಿ ನಿಮಗೆ ಅರ್ಥವಾಗದ ಪ್ರಶ್ನೆಗಳಿದ್ದರೆ ಸಂಕೋಚಿಸದೆ ಪ್ರಶ್ನಿಸಿದಲ್ಲಿ ನಾನು ನಿಮ್ಮ ಪ್ರಶ್ನೆಗೆ ಸಂತೋಷದಿಂದ ಉತ್ತರ ನೀಡುತ್ತೇನೆ. ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳುವುದಕ್ಕೆ ಯಾವುದೇ ರೀತಿಯ ಪರಿಹಾರ ನೀಡಿಲಾಗುವುದಿಲ್ಲ.

ನಿಮಗೆ ಸಂಶೋಧನೆಯಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ಇಷ್ಟವಿರದಿದ್ದರೆ ಅಥವಾ ಯಾವುದೇ ರೀತಿಯ ಅಹಿತಕರ ಘಟನೆಯ ಅನುಭವವಾದರೆ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳುವುದರಿಂದ ಹೊರಗುಳಿಯುವ ಮುಕ್ತ ಅವಕಾಶ ನಿಮಗಿದೆ.

ಮುಖ್ಯ ಸಂಶೋಧಕರ ಹೆಸರು ಮತ್ತು ವಿಳಾಸ:

ಡಾ॥ ಆನಂದ್

ಪಿ,ಎಚ್.ಡಿ ಪದವಿ ವಿದ್ಯಾರ್ಥಿ,

ಜೀವರಸಾಯನ ಶಾಸ್ತ್ರ ವಿಭಾಗ,

ಶ್ರೀ ಬಿ.ಎಂ. ಪಾಟೀಲ್ ವೈದ್ಯಕೀಯ ಕಾಲೇಜು ಮತ್ತು ಆಸ್ಪತ್ರೆ,

ವಿಜಯಪುರ - ೫೮೬೧೦೩, ಕರ್ನಾಟಕ ರಾಜ್ಯ.

ಮೊಬೈಲ್ ಸಂಖ್ಯೆ : ೭೨೫೯೬೭೮೮೦೭.

ANNEXURE – III

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

B.L.D.E. UNIVERSITY

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act,1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

IEC Ref No, 01/2012/dt/10/12/12

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on 21-11-2012 *at* 11 AM *to scrutinize the Synopsis / Research projects of Postgraduate student / Undergraduate student / Faculty members of this University / college from ethical clearance point of view. After scrutiny the following original / corrected & revised version synopsis of the Thesis / Research project has been accorded Ethical Clearance.*

Title "Heart-Type Fatty acid - Binding Protein, in Early detection of Acute Myocardial Infarction Comparison with CK-MB, Troponin I and Myoglobin"

Name of P. G. / U. G. Student / Faculty member Dr Anand
Asst Professor Dept. of Bio-chemistry.

Name of Guide Dr. Dilip. B. Ratni

Dr. Sharada Metgud
Chairperson, I.E.C
BLDE University,
BIJAPUR – 586 103

Mr.G.V.Kulkarni
Secretary,I.E.C
BLDE University,
BIJAPUR – 586 103.

Institutional Ethical Committee
BLDEU's Shri B.M. Patil
Medical College,BIJAPUR-586103.

Following documents were placed before Ethical Committee for Scrutinization:

- Copy of Synopsis / Research project
- Copy of informed consent form
- Any other relevant document's

Smt. Bangaramma Sajjan Campus, Sholapur Road, Bijapur – 586103, Karnataka, India.
University: Phone: +918352-262770, Fax: +918352-263303, Website: www.bldeuniversity.org, E-mail: office@bldeuniversity.org
College: Phone: +918352-262770, Fax: +918352-263019, Website: www.bldea.org, E-mail: bmpmc1@yahoo.co.in

BLDE (DEEMED TO BE UNIVERSITY)

Annexure -I

PLAGARISM VERIFICATION CERTIFICATE

1. **Name of the Student:** DR. ANAND ..... **Reg No.** 11PHD001
2. **Title of the Thesis:** Heart type of fatty acid binding protein, in early detection of acute myocardial infarction.- Comparison with CK-MB, Troponin I and myoglobin.
3. **Department:** Biochemistry
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ANNEXURE – V

PUBLICATIONS

1. Anand K Pyati, Basavaraj B Devaranavadagi, Sanjeev L Sajjannar, Shashikant V Nika m, Mohd Shannawaz, Sudharani. Heart-Type Fatty Acid Binding Protein: A Better Cardiac Biomarker than CK-MB and Myoglobin in the Early Diagnosis of Acute Myocardial Infarction. Journal of Clinical and Diagnostic Research. 2015; 9(10): BC08-BC11.

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Biochemistry Section

Heart-Type Fatty Acid Binding Protein: A Better Cardiac Biomarker than CK-MB and Myoglobin in the Early Diagnosis of Acute Myocardial Infarction

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ABSTRACT

Background: Early diagnosis and therapeutic intervention can improve the outcome of acute myocardial infarction (AMI). However, there are no satisfactory cardiac biomarkers for the diagnosis of AMI within 6 hours of onset of symptoms. Among novel biochemical markers of AMI, heart-type fatty acid binding protein (H-FABP) is of particular interest.

Aim: To compare the diagnostic value of H-FABP with that of CK-MB and myoglobin in suspected AMI patients within first 6 hours after the onset of symptoms.

Settings and Design: The study includes 40 AMI cases and 40 non-cardiac chest pain otherwise healthy controls. The cases and controls were further divided into 2 groups depending on the time since chest pain as those subjects within 3 hours and those between 3-6 hours of onset of chest pain.

Materials and Methods: In all the cases and controls, serum H-FABP, CK-MB and myoglobin concentrations were measured by Immunoturbidimetric method, immuno-inhibition method and Chemiluminescence immunoassay respectively.

Statistical Analysis: Data is presented as mean ± SD values. Differences between means of two groups were assessed by Student *t*-test. Sensitivity, Specificity, Positive predictive value, Negative predictive values were calculated and ROC curve analysis was done to assess the diagnostic validity of each study parameter.

Results: The sensitivity, specificity, PPV, NPV of H-FABP were greater than CK-MB and myoglobin and ROC curve analysis demonstrated highest area under curve for H-FABP followed by myoglobin and CK-MB in patients with suspected AMI both within 3 hours and 3-6 hours after the onset of chest pain.

Conclusion: The diagnostic efficiency of H-FABP is greater than CK-MB and myoglobin for the early diagnosis of AMI within first 6 hours of chest pain. H-FABP can be used as an additional diagnostic tool for the early diagnosis of AMI.

Keywords: Chest pain, Diagnostic validity, Immunoturbidimetric method, ROC analysis, Sensitivity

INTRODUCTION

Among the patients attending the Emergency Department, chest pain is one of the most common and important complaints. Apart from AMI, gastro-oesophageal, musculo-skeletal and pulmonary disorders are the other common causes of chest pain [1]. AMI accounts for 5-15% of all the causes of chest pain in USA. In countries like India, considerably higher number of patients present with chest pain as the chief complaint to the emergency department [2]. By accurately ruling out chest pain of cardiac origin, 40% of patients presenting with acute chest pain could be spared from the risks and costs of unnecessary hospital admission and more invasive cardiac testing [3]. Early accurate diagnosis and treatment of acute myocardial infarction (AMI) can reduce the mortality and associated long term morbidity. Majority of deaths due to AMI occur during the first hour after the onset of symptoms. If AMI cases are diagnosed and treated effectively during the first hour (so called Golden Hour) after the onset of symptoms, the mortality can be reduced from 9% to 3%, but if delayed for 3-4 hours mortality can be 5 times higher [4].

However, there are no reliable tests for the diagnosis of AMI in the early hours after the onset of symptoms. Electrocardiographic (ECG) ST elevation has only 50-60% sensitivity for the diagnosis of myocardial necrosis [5]. Currently creatine kinase–MB isoenzyme (CK-MB), myoglobin and cardiac troponins (cTnI & cTnT) are used in the diagnosis of AMI. However, these cardiac marker proteins are also not satisfactory for the diagnosis of AMI within first 6 hours of the onset of chest pain. Myoglobin which appears in the blood within 2 hours after myocardial infarction, lacks specificity because myoglobin released from skeletal muscles cannot be distinguished from that released from heart. Cardiac troponins and CK-MB though more specific for cardiac injury, lack early sensitivity because their blood concentrations do not rise until 6-8 hours after the onset of symptoms [6].

Heart-type fatty acid binding protein (H-FABP) is a novel marker with the potential for the early diagnosis of AMI within 6 hours of onset of symptoms. It offers several theoretical advantages over traditional cardiac bio-markers. H-FABP is a 15 kDa soluble protein, consisting of 132 amino acids. It is one of the most abundant proteins in the cardio myocytes comprising 5-15% of the total cytosolic protein pool. It is involved in the delivery of fatty acyl coenzyme A for oxidation in the mitochondria. Under normal conditions H-FABP is not present in plasma. During ischemia, H-FABP leaks out of myocardial tissue and the concentration increases in the blood within 2 hours and is reported to peak at about 4-6 hours and return to normal baseline value in 20 hours [7].

In view of above observations, we compared the diagnostic value of H-FABP with CK-MB and myoglobin within 6 hours after the onset of symptoms. The objective of the study was to compare the sensitivity, specificity, positive predictive value, negative predictive value and area under ROC curve of H-FABP with those of myoglobin and CK-MB within 0-3 hours and 3-6 hours after the onset of chest pain.

MATERIALS AND METHODS

The study was carried out at BLDEU's Shri B M Patil Medical College, Hospital and Research Centre, Vijayapur, Karnataka, India during the period from May 2013 to June 2014. The study was approved by the Institutional Ethical Clearance Committee (IECC) and informed consent was obtained from all the study participants

before their inclusion in the study. Also the procedures followed were in accordance with the Helsinki Declaration of 1975 that was revised in 2000.

The study included 40 AMI cases and 40 non cardiac chest pain but otherwise healthy controls. Both cases and controls were further divided into two groups as those reporting within 3 hours and those between 3-6 hours after the onset of chest pain. As a part of routine assessment in our institution each patient underwent an initial clinical and laboratory evaluation, which included the detailed clinical history, clinical examination, standard 12 lead ECG, chest X-ray, routine blood investigations, echocardiography and cardiac biomarkers (CK-MB and cardiac troponin I). Diagnosis of either MI or non-cardiac chest pain was made after critical review of all the above information by a cardiologist. Patients arriving to hospital after 6 hours of onset of chest pain, those with Chronic muscle disease, renal disease, recent surgery, those receiving direct current shocks and who underwent PTCA or CABG procedures within 30 days were excluded from the study.

From all the cases and controls, 5 ml of venous blood sample was drawn as early as possible after admission using all the aseptic precautions, serum was separated and kept at -20^0 C until the analysis was done. Serum troponin-I [8] and myoglobin [9] levels were measured by chemiluminescence immunoassay on Abbott Architect c4000 analyser (Abbott Laboratories, Illinois, USA) and serum CK-MB by Immuno-inhibition method [10,11] using Stat Fax 3300 Biochemistry analyser (Awareness Technology Inc., Florida, USA). Serum H-FABP levels were measured by Automated Immuno-turbidimetric method [12,13] (Reagent kit from Randox Laboratories, and using Roche C-311 fully automated Biochemistry analyser). The method is based on the principle that, H-FABP present in the sample reacts with buffer and anti-H-FABP coated latex. The formation of antigen-antibody complex during the reaction results in an increase in turbidity, the extent of which is measured as the amount of light absorbed at 700 nm. By constructing a standard curve from the absorbance of the standards, H-FABP concentration in the sample can be determined. The cut off levels and coefficients of variation (CV) of H-FABP, troponin-I, myoglobin and CK-MB used for the diagnosis of AMI in this study were >6.32ng/ml (at 99th percentile & CV = 7.94%), >0.032 µg/L in males & >0.022 µg/L in females (at 99th percentile & CV = 10%), >106 ng/ml in males & >155 ng/ml in females (at 99th percentile & CV = 7.94%) and 24 IU/L respectively. These values are according to the recommendations of the reagent kit manufacturers [8-13].

STATISTICAL ANALYSIS

Data is presented as mean ± SD values. Differences between means of two groups were assessed by Student *t*-test. Sensitivity, specificity, positive and negative predictive values were calculated and Receiver operating characteristic (ROC) curve analysis was done to assess the diagnostic accuracy of each study parameter. For all the tests, p-value of 0.05 or less is considered for statistical significance.

RESULTS

A total of 80 patients were included in this study. Of these 40 were AMI cases and 40 were non cardiac chest pain otherwise healthy controls. Of 40 AMI cases 28 were males and 12 females and of 40 controls 27 were males and 13 females. The average age for the AMI cases was 60.45 years and that in controls was 54.83 years. [Table/Fig-2] shows the mean levels of four cardiac biomarkers in AMI cases and controls. The mean levels of H-FABP, myoglobin, troponin I and CK-MB activity were significantly higher in AMI cases when compared to that of controls in both 0-3 hour and 3-6 hour groups. In both the groups, the difference was statistically significant (p < 0.01). [Table/Fig-3] shows the sensitivity, specificity, positive and negative predictive values of H-FABP were greater than CK-MB and myoglobin in both 0-3 hour and 3-6 hour groups. The

Sl No.	Clinical characteristic	AMI cases (n = 40)	Controls (n = 40)
01	Age (Years) (Mean ± SD)	60.5 ± 12.8	54.8 ± 15.2
02	Males	28 (70%)	27 (67.5%)
03	Females	12 (30%)	13 (32.5%)
04	Smoking	12 (30%)	11 (27.5%)
05	Alcohol intake	11 (27.5%)	11 (27.5%)
06	Hypertension	17 (42.5%)	10 (25%)
07	Diabetes Mellitus	13 (32.5%)	10 (25%)
08	Hyperlipidemia	06 (15%)	03 (7.5%)
09	H/o previous IHD	02 (5%)	00
10	ECG changes • ST Elevation • T- wave inversion • Other changes (ST depression/ Tall T waves/ Q wave)	 33 (82.5%) 07 (17.5%) 07 (17.5%)	 -- 05 (8.3%) 03 (5%)
11	Troponin I (µg/L) (Mean ± SD)	7.57 ± 16.9	0.0065 ± 0.008

[Table/Fig-1]: Baseline clinical characteristics of AMI cases and controls

	AMI cases (Mean ± SD)		Controls (Mean ± SD)			
	0-3 h (n = 17)	3-6 h (n = 23)	0-3 h (n = 17)	3-6 h (n = 23)	p1 value	p2 value
CK-MB (IU/L)	40.9 ± 22.6	74.9 ± 58.4	19 ± 10.04	25.2 ± 10.1	< 0.01	< 0.01
Myoglobin (ng/mL)	320.1 ± 344.7	439.9 ± 396.7	60.96 ± 57.7	87.1 ± 75.4	< 0.01	< 0.01
H-FABP (ng/mL)	31.9 ± 51.7	66.4 ± 58.1	5.28 ± 8.5	5.9 ± 7.8	< 0.01	< 0.01
Troponin I (µg/L)	10.4 ± 16.5	12.4 ± 17.5	0.005 ± 0.008	0.008 ± 0.008	< 0.01	< 0.01

[Table/Fig-2]: Mean levels of four cardiac biomarkers in cases and controls
p_1 = AMI cases (0-3 hours group) versus Controls (0-3 hours group)
p_2 = AMI cases (3-6 hours group) versus Controls (3-6 hours group)

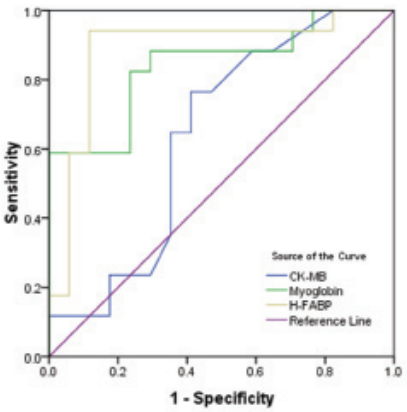
	Sensitivity		Specificity		PPV		NPV	
	0-3 h	3-6 h	0-3 h	3-6 h	0-3 h	3-6 h	0-3 h	3-6 h
CK-MB (IU/L)	23.5	60.9	58.8	56.5	36.4	58.3	43.5	59.1
Myoglobin (ng/mL)	70.6	87	76.5	69.6	75	74	72.2	84.2
H-FABP (ng/mL)	94.1	91.3	88.2	73.9	88.9	77.8	93.8	89.5

[Table/Fig-3]: Sensitivity, specificity, PPV and NPV of CK-MB, myoglobin and H-FABP in 0-3 hour and 3-6 hour groups after the onset of chest pain
PPV = Positive Predictive Value; NPV = Negative Predictive Value

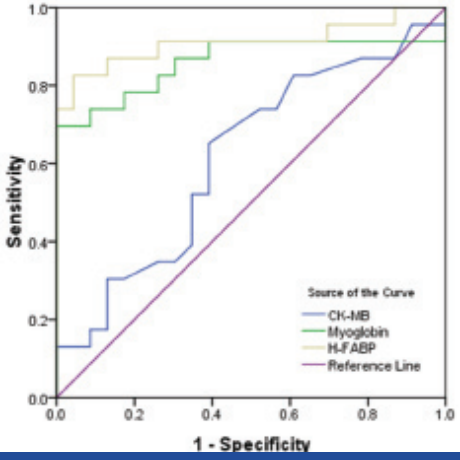
diagnostic ability of each marker to distinguish between AMI and non-AMI groups was assessed by receiver operating characteristic curve (ROC) analysis. The area under the curve (AUC) of H-FABP was higher than CK-MB and myoglobin in both 0-3 hour group and 3-6 hour groups [Table/Fig-4-7].

DISCUSSION

Acute myocardial infarction is the major cause of mortality and long term morbidity in the modern world. Early and correct diagnosis is of utmost importance to enable the immediate and intensified treatment which consequently reduces the mortality [14]. The golden time for the optimal outcome of coronary revascularization is within 4 hours after the onset of coronary thrombosis [15]. Cardiac troponins, CK-MB and myoglobin assays are the most commonly used cardiac markers for the detection of AMI [16]. Collisson et al., showed that, diagnosis of AMI cannot be made solely on the basis of either CK-MB or troponin I individual test results [17]. Yamamoto et al., demonstrated that, rapid quatitative test for cTnT and myoglobin at 6, 12, 24, 48 hours after onset of symptoms is useful for the early diagnosis and assessment of severity in AMI [18]. To date, myoglobin is the only cardiac



[Table/Fig-4]: ROC curve analysis of CK-MB, Myoglobin & H-FABP in 0-3 hour group



[Table/Fig-5]: ROC curve analysis of CK-MB, Myoglobin & H-FABP in 3-6 hour group

	AUC	p-value	Confidence Interval	
			Lower limit	Upper limit
CK-MB	0.640	0.163	0.446	0.834
Myoglobin	0.841	0.001	0.705	0.977
H-FABP	0.886	0.000	0.756	1.015

[Table/Fig-6]: Area under ROC curve for cardiac markers in 0-3 hour group
AUC = Area under curve

	AUC	p-value	Confidence Interval	
			Lower limit	Upper limit
CK-MB	0.616	0.177	0.452	0.781
Myoglobin	0.860	0.000	0.738	0.983
H-FABP	0.911	0.000	0.816	1.007

[Table/Fig-7]: Area under ROC curve for cardiac markers in 3-6 hour group
AUC = Area under curve

biomarker used for the diagnosis of AMI in hyperacute phase (within 3 hours after the onset of symptoms). However, myoglobin is not a specific marker as its concentration is much higher in skeletal muscles than cardiac tissue [19].

In the present study, the incidence of myocardial infarction was more common in males, smokers, and those having hypertension, diabetes and hyperlipidemia. The reason being the above mentioned factors are the known risk factors for coronary heart disease [20]. In cases diagnosed with AMI, only 83.5% of the patients were having ECG changes and among the control group, 8.3% of the patients with non-cardiac chest pain presented with ECG changes suggestive of AMI. The diagnostic value of the admission ECG is limited in following situations; i) The presence of conduction disorders including left bundle branch block (LBBB); ii) If Q waves and ST-T changes are already present e.g. old infarcts and digoxin effects, respectively; iii) ST-T wave changes of marked left ventricular hypertrophy; iv) In posterior infarct or right ventricular infarct. 30% of patients may have no diagnostic changes on their admission ECG [7].

In our study, mean serum concentrations of HFABP, myoglobin and CK-MB activity were significantly higher ($p< 0.01$) in AMI cases when compared to controls both in 0-3 hour and 3-6 hour groups [Table/Fig-2]. Also, the mean levels of cardiac markers were much higher in 3-6 hour group when compared to 0-3 hour group of AMI cases. This finding is in full agreement with the studies conducted by Glatz JFC et al., Elmadbouh I et al., Pasaoglu H et al., and Orak M et al., [21-24].

In this study, H-FABP demonstrated highest sensitivity, specificity, PPV and NPV followed by myoglobin and CK-MB in patients with suspected AMI both within 3 hours and 3-6 hours after the onset of chest pain [Table/Fig-3]. This finding emphasizes two important facts: High sensitivity is essential for the early 'rule in' of AMI patients and high NPV is important for the early 'rule out' of AMI. Since more than 90% of patients who present with acute chest pain to an emergency department do not have AMI, H-FABP can prevent unnecessary admissions or inadvertent discharge of patients suspected for AMI.

Many studies have compared the diagnostic efficiency of H-FABP with the two routinely used cardiac markers CK-MB and myoglobin in the early diagnosis of AMI and arrived at nearly consistent results. In their study, Glatz JFC et al., found H-FABP having greater diagnostic sensitivity (73% at 1.5-3 hour and 100% at 4.5-6 hours) than myoglobin and CK-MB in the early diagnosis of AMI [21]. Elmadbouh I et al., found that within 3 hours, H-FABP had diagnostic sensitivity (81.8%) equal to that of CK-MB [22]. However, H-FABP had higher specificity (88.2%) equal to that of myoglobin but superior to that of CK-MB. This trend extends to within 6 hours as well. H-FABP had highest PPV (81.8%) and NPV (88.2%) [12]. Pasaoglu H et al., demonstrated that at 1-2, 3 and 6 hours after the onset of AMI, the diagnostic sensitivity and specificity of H-FABP were higher than CK-MB but similar to myoglobin [23]. In another study Orat M et al., showed that, for patients admitted with chest pain, HFABP is more sensitive and specific than CK-MB in the early diagnosis of ACS [24]. In patients with ACS who were admitted within 0 to 3 hours, the H-FABP sensitivity was 100% and specificity was 75%; and for CK-MB, the sensitivity was 81% and the specificity was 16%. In patients with ACS who were admitted within 3 to 6 hours, the H-FABP sensitivity was 97% and specificity was 68%; and for CK-MB, the sensitivity was 90% and specificity was 80% [14]. Alhadi H et al., conducted a study in 100 consecutive patients admitted with acute chest pain suggestive of ACS and found that, H-FABP peak concentration occurred at 8 hours after symptoms onset and was the most sensitive early marker with 79.9%, 98% and 95.3% sensitivity at presentation, 2 hours and 4 hours after presentation respectively [25]. The sensitivity of all other cardiac markers (CK-MB mass and myoglobin) was <62% at presentation. The negative predictive value of H-FABP was also superior to other markers. Myoglobin was the second most sensitive early marker at presentation. McMahon CG et al., concluded that, of the four biomarkers measured, H-FABP demonstrated highest sensitivity (64.3% at 0-3 hours and 85.3% at 3-6 hours) and NPV (93% at 0-3 hours and 97% at 3-6 hours) at the early time points [26].

Diagnostic ability of each cardiac marker for 0-3 hours and 3-6 hours groups were examined by ROC curve analysis. In both the groups, H-FABP had the highest area under curve followed by myoglobin and CK-MB [Table/Fig-4,5]. The AUC values of these markers in both the groups were statistically significant ($p < 0.05$) except for CK-MB [Table/Fig-6,7]. This finding is in agreement with the studies conducted by Elmadbouh I et al., Pasaoglu H et al., Orak M et al., McMahon CG et al., and Kim KS et al., [22-24,26,27].

The observed better diagnostic value of H-FABP than myoglobin and CK-MB in AMI is probably because of its early rise in the serum concentration which in turn is related to the smaller molecular size (15kDa and 17 kDa for H-FABP and myoglobin respectively), higher concentration in the myocardial tissue (Concentration of myoglobin is approximately 2 fold lower in cardiac than skeletal muscle but H-FABP concentrations are 2-10 fold higher in heart than in skeletal muscle) and very low plasma concentration under normal conditions (< 5 µg/l; The ratio of cytoplasmic to vascular concentration is 200000:1, which is 10-15 fold lower than myoglobin). Even though H-FABP is not completely cardiac specific, its tissue distribution outside the heart is comparable to that of CK-MB [7]. CK-MB has low sensitivity because it takes atleast 4-6 hours after the onset of myocardial injury to appear in the blood. Due to outstanding diagnostic performance of cardiac troponins, H-FABP is likely to play a role only in the early presentation after the symptom onset.

Limitations for the clinical use of H-FABP concentration in the early diagnosis of AMI includes: Increase in the concentration of H-FABP may be seen in conditions of skeletal muscle injury (e.g. intramuscular injections, electric cardioversion, traumatic cardiopulmonary resuscitation, surgery, crush injury, etc.) as the FABP from skeletal muscle origin is identical to that of cardiac origin. Also, renal insufficiency can result in the elevation of H-FABP level because it is excreted thoursough the kidney [7].

LIMITATIONS

Our study presents few limitations such as small sample size and we could not do the serial measurements of the cardiac markers for the assessment of their kinetics.

CONCLUSION

H-FABP is more sensitive and specific cardiac biomarker than myoglobin and CK-MB and shows better diagnostic efficiency for the early diagnosis of myocardial infarction within 6 hours of chest pain. H-FABP can be used as an additional diagnostic tool for the early diagnosis of acute myocardial infarction.

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Heart-Type Fatty Acid-Binding Protein, in Early Detection of Acute Myocardial Infarction: Comparison with CK-MB, Troponin I and Myoglobin

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ORIGINAL ARTICLE

Heart-Type Fatty Acid-Binding Protein, in Early Detection of Acute Myocardial Infarction: Comparison with CK-MB, Troponin I and Myoglobin

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Abstract The study aimed to investigate whether heart-type fatty acid binding protein (H-FABP) measurement provides additional diagnostic value to that of conventional cardiac markers in acute myocardial infarction (AMI) within first 6 h after the onset of symptoms. The study included 120 subjects: 60 AMI cases and 60 age and sex matched controls. The cases and controls were further divided into 2 subgroups depending on the time since onset of chest pain as (1) subjects within 3 h and (2) between 3 and 6 h of onset of chest pain. In all the cases and controls, serum H-FABP concentration was measured by Immunoturbidimetric method, serum Troponin I and myoglobin concentrations by Chemiluminescence immunoassay and serum CK-MB concentration by Immuno-inhibition method. The sensitivity, specificity, positive and negative predictive values of H-FABP were significantly greater than CK-MB and myoglobin but were lesser than Troponin I in patients with suspected AMI in both within 3 h and 3–6 h groups. Receiver operating characteristic curves demonstrated greatest diagnostic ability for Troponin I (AUC = 0.99, $p < 0.001$) followed by H-FABP (AUC = 0.906, $p < 0.001$) within 3 h and 3–6 h after the onset of chest pain. In conclusion, the diagnostic value of H-FABP is greater than CK-MB and myoglobin but slightly lesser than troponin I for the early diagnosis of AMI within first 6 h of chest pain. H-FABP can be used as an additional diagnostic tool for the early diagnosis of AMI along with troponin I.

Keywords Myocardial infarction · Heart-type fatty acid binding protein, human · Troponin-I · Myoglobin · Creatine kinase, MB · Immunoturbidimetric method · ROC analysis

Introduction

Acute coronary syndrome (ACS) is a group of heart conditions caused by insufficient blood supply to the heart. ACS includes acute myocardial infarction (AMI), when blood flow to part of the heart is blocked for long enough that part of the heart muscle is damaged or dies [1]. AMI is one of the leading causes of death in India and worldwide. AMI prevalence rates range from 1.6 to 7.4 % in rural populations and 1 to 13.2 % in urban populations in India [2].

The early diagnosis and treatment is of utmost importance to prevent the mortality and related long term complications. If AMI is diagnosed and treated within 1 h (so called golden hour), mortality can be reduced from 9 to 3 %, and if the treatment is delayed for 3–4 h, mortality could be 5 times higher. Unfortunately, at least one-fifth of the cases of AMI go unrecognized either because of the atypical presentation or atypical ECG changes or delay in the rise of the serum cardiac markers [3].

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Currently used biochemical markers such as cardiac troponins, CK-MB or myoglobin are limited by either the lack of specificity or delay in their elevation in the blood by several hours after the onset of symptoms. Therefore their use in the diagnosis of AMI in the early hours (within 6 h) is limited [4]. Among the novel biochemical markers of AMI, heart-type fatty acid binding protein (H-FABP) is of particular interest. H-FABP is a small 15 kDa soluble protein composed of 132 amino acids. It is one of the most abundant proteins in the heart comprising 5–15 % of the total cytosolic protein pool. H-FABP is not totally cardiac specific, occurs in other tissues at much lower concentrations. It is involved in intracellular transportation of fatty acids for oxidation in the mitochondria. Several studies have shown that H-FABP is a sensitive marker for the diagnosis of AMI and might be more sensitive than conventional cardiac markers when measured soon after the onset of symptoms. However, there is still considerable uncertainty regarding the role of H-FABP in the early diagnosis of AMI. Use of H-FABP is restricted to the clinical research because of lack of fast and easy to use method for its measurement. However, a novel automated immuno-turbidimetric H-FABP assay has recently been developed [5].

Aim of this study was to assess whether H-FABP measurement provides additional diagnostic value to that of conventional cardiac markers in AMI. Objective of the study was to compare the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic ability of H-FABP with the conventional cardiac biomarkers (CK-MB, Troponin I and Myoglobin) in patients presenting with chest pain within 3 h and 3–6 h after the onset of chest pain.

Materials and Methods

The study was carried out on 120 subjects: 60 MI patients and 60 non-cardiac chest pain but otherwise healthy controls. Patients were selected from ICCU, BLDEU's Shri B M Patil Medical College, Hospital and Research Centre, Vijayapur, during the period from May 2013 to June 2014. The study was approved by the Institutional Ethical Clearance Committee and written informed consent was obtained from all the subjects. All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Patients presenting with acute chest pain less than 6 h duration suspected of AMI were enrolled consecutively in the study. Full clinical history including personal information, presenting symptoms, past medical history such as diabetes mellitus, hypertension, previous ischemic event etc., and general clinical examination findings, ECG findings, Echocardiography and laboratory investigations were documented using a predefined protocol. Standard diagnosis of either AMI or non-cardiac chest pain was made after critical review of all the above information by a cardiologist. Diagnosis of AMI was done according to the WHO criteria. The cases and controls were further divided into two subgroups depending on the time since the onset of chest pain as the subjects within 3 h of onset of chest pain and those between 3 and 6 h of onset of chest pain. Patients arriving to hospital after 6 h of onset of chest pain, those with chronic muscle disease, renal disease, recent surgery, those receiving direct current shocks and who underwent percutaneous transluminal coronary angioplasty (PTCA) or coronary artery bypass graft (CABG) procedures within 30 days were excluded from the study.

From all the cases and controls, 5 mL of venous blood sample was drawn using all the aseptic precautions, serum was separated and kept at −20 °C until the analysis was done. Serum H-FABP levels were measured by automated immuno-turbidimetric method [6–8] (Reagent kit from Randox Laboratories Limited, County Antrim, United Kingdom) on Roche cobas c 311 fully automated Biochemistry analyzer (Roche Diagnostic Limited, Rotkreuz, Switzerland). The method is based on the principle that, sample is reacted with a buffer and anti-H-FABP coated latex. The formation of antigen–antibody complex during the reaction results in an increase in turbidity, the extent of which is measured as the amount of light absorbed at 700 nm. By constructing a standard curve from the absorbance of the standards, H-FABP concentration in the sample can be determined. Serum troponin-I [9] and myoglobin [10] levels were measured by chemiluminescence immunoassay on Abbott Architect c4000 analyzer (Abbott Laboratories, Illinois, USA) and serum CK-MB by Immuno-inhibition method [11, 12] using Stat Fax 3300 Biochemistry analyzer (Awareness Technology Inc., Florida, USA).

The cut off levels and coefficients of variation (CV) of H-FABP, troponin-I, myoglobin and CK-MB used for the diagnosis of AMI in this study were >6.32 ng/mL (at 99th percentile, CV = 7.94 %), >0.032 μg/L in males and >0.022 μg/L in females (at 99th percentile, CV = 10 %), >106 ng/mL in males and >155 ng/mL in females (at 99th percentile, CV = 7.94 %) and 24 IU/L respectively. These values are according to the recommendations of the reagent kit manufacturers.

Statistical Analysis

Continuous variables are presented as mean ± standard deviation (SD) values and categorical data are presented as

percent frequency of occurrence. Differences between means of two groups were assessed with the unpaired Student *t* test. Sensitivity, Specificity, positive and negative predictive values (PPV and NPV) were calculated and receiver operating characteristic (ROC) curve analysis was done to assess the diagnostic validity for each marker at each time interval. For all the tests, p value of 0.05 or less was considered for statistical significance. All the statistical procedures were performed using the Statistical Package for the Social Sciences statistical software (SPSS) version 16 for Windows.

Results

A total of 120 patients were included in this study. Of these 60 were AMI cases and 60 were non-cardiac chest pain otherwise healthy controls. Of 60 AMI cases 44 were males and 16 females and of 60 controls 38 were males and 22 females. The average age for the AMI cases was 60.7 years and that in controls was 54.82 years.

The baseline clinical characteristics are depicted in Table 1. Patients diagnosed with AMI tended to be older than non-cardiac chest pain controls. AMI was more common among males when compared to females. The other demographics such as incidence of smoking, alcohol intake, hypertension, diabetes mellitus, hyperlipidemia, H/o previous IHD was more common in AMI cases when compared to controls. Among the ECG changes, ST elevation was seen among 85 % of AMI cases, T wave inversion in 20 % and other changes in 8.3 % of the AMI cases.

Table 2 shows the mean ± SD values of four cardiac biomarkers in AMI cases and non-AMI controls. The mean levels of CK-MB, troponin I, myoglobin and H-FABP were significantly higher in AMI cases when compared to that of controls in both 0–3 and 3–6 h groups. The difference between cases and controls was statistically significant in both the time intervals ($p < 0.01$).

Table 3 shows the sensitivity, specificity, PPV and NPV of all the four cardiac biomarkers. As it is evident from the table that, the sensitivity, specificity, PPV and NPV of H-FABP were significantly higher than that of CK-MB and myoglobin and slightly lesser than that of troponin I in both 0–3 h and 3–6 h groups.

Within 3 h after the onset of chest pain, ROC curve analysis of CK-MB, Troponin I, Myoglobin and H-FABP shows that, troponin I has highest diagnostic accuracy (AUC = 0.997) followed by H-FABP (AUC = 0.886), myoglobin (AUC = 0.841) and CK-MB (AUC = 0.64) (Fig. 1).

Between 3 and 6 h after the onset of chest pain, ROC curve analysis of CK-MB, Troponin I, Myoglobin and H-FABP shows that, troponin I has highest diagnostic accuracy (AUC = 0.982) followed by H-FABP (AUC = 0.911), myoglobin (AUC = 0.86) and CK-MB (AUC = 0.616) (Fig. 2).

Discussion

In our study, serum concentrations of HFABP, troponin-I, myoglobin and CK-MB were significantly higher in AMI cases when compared to controls both in 0–3 h and 3–6 h groups. These findings are in full agreement with the study conducted by Elmadbouh et al. [3]. Pasaoglu et al. [13] showed that, in blood samples collected at 1–2 and 3 h,

Table 1 Baseline characteristics of study participants

Clinical characteristic	AMI cases (n = 60)	Controls (n = 60)
Age (years) (mean ± SD)	60.7 ± 12.2	54.82 ± 14.4
Males	44 (73.3 %)	38 (63.3 %)
Females	16 (26.7 %)	22 (36.7 %)
Smoking	18 (30 %)	16 (26.7 %)
Alcohol intake	16 (26.7 %)	16 (26.7 %)
Hypertension	25 (41.7 %)	15 (25 %)
Diabetes mellitus	19 (31.7 %)	15 (25 %)
Hyperlipidemia	09 (15 %)	05 (8.3 %)
H/o previous IHD	03 (5 %)	00
ECG changes		
ST elevation	51 (85 %)	–
T-wave inversion	12 (20 %)	05 (8.3 %)
Other changes (ST depression/tall T waves/Q wave)	05 (8.3 %)	03 (5 %)

Values are given as 'n' (%), unless otherwise specified

IHD ischemic heart disease

Table 2 Mean levels of four cardiac biomarkers in AMI cases and controls

	AMI cases (n = 60) (Mean ± SD)		Controls (n = 60) (Mean ± SD)		P_1 value	P_2 value
	0–3 h (n = 26)	3–6 h (n = 34)	0–3 h (n = 26)	3–6 h (n = 34)		
CK-MB (IU/L)	26.2 ± 19.2	36.9 ± 48.9	18.6 ± 9.5	25 ± 10.1	<0.01	<0.01
Troponin I (μg/L)	5.9 ± 16.2	8.5 ± 16.9	0.0048 ± 0.0077	0.0087 ± 0.0079	<0.01	<0.01
Myoglobin (ng/mL)	364.9 ± 363.9	584.5 ± 393.9	55.7 ± 53.4	90.8 ± 74.2	<0.01	<0.01
H-FABP (ng/mL)	40.4 ± 56.3	76.3 ± 56.1	4.7 ± 7.3	6.7 ± 8.6	<0.01	<0.01

P_1 = AMI cases (0–3 h group) versus controls (0–3 h group)

P_2 = AMI cases (3–6 h group) versus controls (3–6 h group)

Table 3 Diagnostic performances of four cardiac biomarkers for the early diagnosis of AMI within 3 h and 3–6 h after the onset of chest pain

	Sensitivity		Specificity		PPV		NPV	
	0–3 h	3–6 h	0–3 h	3–6 h	0–3 h	3–6 h	0–3 h	3–6 h
CK-MB (IU/L)	23.1	52.9	61.5	58.8	37.5	56.3	44.5	55.6
Troponin I (μg/L)	96.2	100	100	100	100	100	96.3	100
Myoglobin (ng/mL)	73.1	88.2	76.9	70.6	76.0	75.0	74.1	85.7
H-FABP (ng/mL)	92.3	94.1	88.5	79.4	88.9	82.1	92.0	93.1

PPV positive predictive value, *NPV* negative predictive value

H-FABP and myoglobin in the AMI group were significantly higher ($p = 0.00$) than in non-AMI and control groups. Troponin-I and CK-MB in the AMI group were not yet significantly higher than in non-AMI and control groups at 1–2 h. Troponin-I in the AMI group was higher than in the non-AMI and control groups at 3 h. CK-MB in the AMI group was higher than in the control group, but not in the non-AMI group at 3 h. All parameters in the AMI group were significantly higher than in the non-AMI and control groups at 6 h [13]. Orak et al. [14] found that, there was a significant difference in the mean levels of H-FABP and CK-MB in ACS cases when compared to the controls ($p = 0.000$), whereas no difference was observed for troponin-I ($p = 0.013$) within 6 h [14].

In our study, H-FABP demonstrated greater sensitivity, specificity, PPV and NPV than CK-MB and myoglobin in patients with suspected AMI both within 3 h and 3–6 h after the onset of chest pain. The diagnostic validity of H-FABP was comparable to troponin-I but never superior both within 3 h and 3–6 h after the onset of chest pain.

Several studies have compared the diagnostic value of H-FABP with CK-MB, myoglobin and troponin-I in patients with chest pain and arrived at conflicting conclusions. In their study, Elmadbouh et al. [3] found that within 3 h, H-FABP had diagnostic sensitivity (81.8 %) equal to that of CK-MB and troponin-I but superior to that of myoglobin (72.7 %). However, H-FABP had higher specificity (88.2 %) equal to that of myoglobin but superior to that of CK-MB and troponin-I. This trend extends to within 6 h as well [3]. Pasaoglu et al. [13] demonstrated that for AMI detection, serum H-FABP shows significantly higher diagnostic sensitivity and specificity than troponin-I and CK-MB, similar to myoglobin, especially soon after (within 1–2 and 3 h) the onset of symptoms [13]. In another study, Orak et al. [14] showed that, for patients admitted with chest pain, H-FABP is more sensitive and specific than troponin-I and CK-MB in the early diagnosis of ACS. In patients with ACS who were admitted within 0–3 h, the H-FABP sensitivity was 100 % and specificity was 75 %; for CK-MB, the sensitivity was 81 % and the specificity was 16 %; and for troponin-I, the sensitivity was 100 % and the specificity was 20 %. In patients with ACS who were admitted within 3–6 h, the H-FABP sensitivity was 97 % and specificity was 68 %; for CK-MB, the

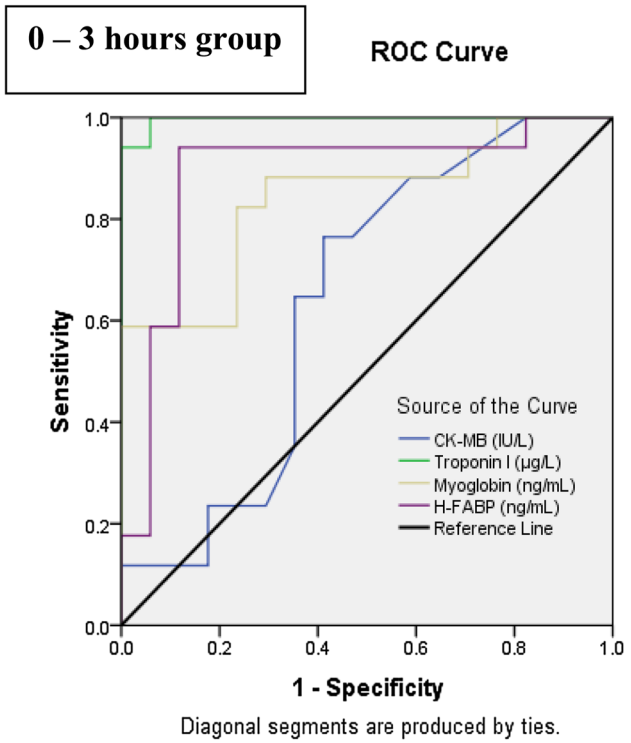

Fig. 1 ROC curve analysis of CK-MB, Troponin I, Myoglobin and H-FABP in 0–3 h group

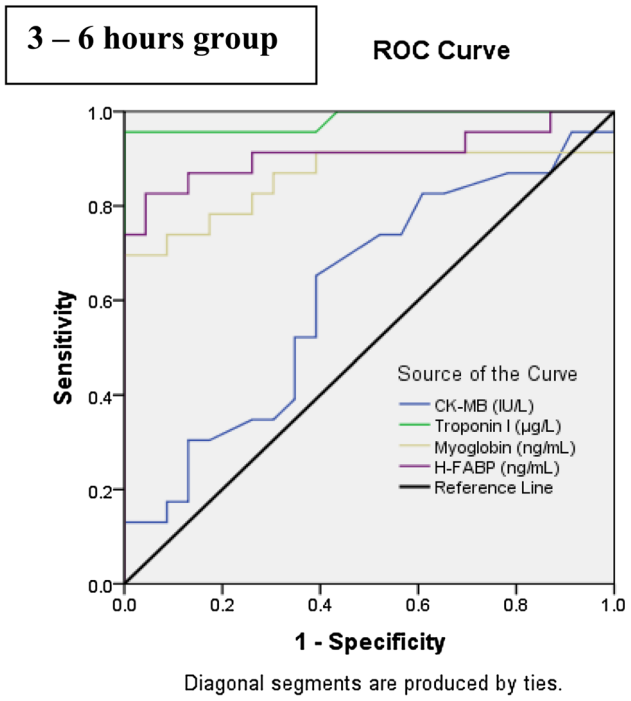

Fig. 2 ROC curve analysis of CK-MB, Troponin I, Myoglobin and H-FABP in 3–6 h group

sensitivity was 90 % and specificity was 80 %; and for troponin-I, the sensitivity was 75 % and specificity was 21 % [14]. Alhadi et al. [5] conducted a study in 100 consecutive patients admitted with acute chest pain suggestive of ACS and found that, H-FABP peak concentration occurred at 8 h after symptoms onset and was the most sensitive early marker with 79.9 and 98 % sensitivity at presentation and 2 h after presentation respectively. The sensitivity of all other markers namely CK-MB, troponin-I and myoglobin at presentation was less than 62 % [15]. McMahon et al. [4] concluded that, of the four biomarkers measured in this study, H-FABP demonstrated highest sensitivity at the early time points (64.3 % at 0–3 h and 85.3 % at 3–6 h) [4].

In contrast, Alansari and Croal [16] suggested that H-FABP and myoglobin provide little clinical value compared to troponin-I, when measured at presentation in patients presenting with chest pain (3–12 h) [16]. In a multicentre study conducted by Freund et al. [17], H-FABP had no additional value over cardiac troponin-I for the diagnosis of myocardial necrosis (STEMI and NSTEMI) in ED patients with chest pain of less than 6 h duration [17].

We examined the diagnostic ability of each cardiac marker for 0–3 and 3–6 h groups by ROC curve analysis. In both 0–3 and 3–6 h groups, troponin I had the highest area under curve value followed by H-FABP, myoglobin and CK-MB. The AUC values of these markers were statistically significant except for CK-MB. This finding is in full agreement with the studies conducted by Freund et al. [17] and Kim et al. [18]. However McMahon et al. and Orak et al. showed that, AUC for H-FABP was highest of all markers at 0–6 h after chest pain followed by CK-MB, troponin I and myoglobin [4] (Tables 4, 5).

Superior performance of the H-FABP over CK-MB and myoglobin may be because of the following reasons: (1) H-FABP has small molecular weight (15 kDa) when compared to CK-MB (80 kDa) and myoglobin (18 kDa). (2) Relative specificity of the H-FABP for the cardiac tissue. Its concentration within the myocardial tissue is 10 times higher when compared to that of skeletal muscle. However, CK-MB and myoglobin are less specific for cardiac tissue; concentration of myoglobin is approximately twofold lower in cardiac than skeletal muscle. (3) H-FABP appears in the plasma early (within 2 h) with a peak at 6 h after the cardiac damage. However CK-MB begins to increase in the blood between 3 and 6 h after the onset of infarction and peaking at 16–20 h [5].

Several studies [3, 4, 13–15] found that the diagnostic value of H-FABP was greater than troponin-I particularly within 3 h after the onset of symptoms of AMI. Some other studies [16–18] showed H-FABP does not provide additional useful information to troponin-I. Reasons for the conflicting results can be the duration of MI at the time of sampling, different study settings (cardiology units, Emergency department or pre-hospital etc.), different methods and cut off values for the H-FABP used.

Table 4 Area under ROC curve for cardiac markers in 0–3 h group

	AUC	*p* value	CI	
			Lower limit	Upper limit
CK-MB	0.640	0.163	0.446	0.834
Troponin I	0.997	0.000	0.985	1.008
Myoglobin	0.841	0.001	0.705	0.977
H-FABP	0.886	0.000	0.756	1.015

AUC area under curve

Table 5 Area under ROC curve for cardiac markers in 3–6 h group

	AUC	*p* value	CI	
			Lower limit	Upper limit
CK-MB	0.616	0.177	0.452	0.781
Troponin I	0.982	0.000	0.946	1.019
Myoglobin	0.86	0.000	0.738	0.983
H-FABP	0.911	0.000	0.816	1.007

AUC area under curve

Limitations

We are aware that our study presents few limitations. First, sample size is small. Second, we could not do the serial measurements of the cardiac markers for the assessment of their kinetics.

Conclusion

Diagnostic value of H-FABP is much better than CK-MB and myoglobin but lesser than troponin I for the early diagnosis of myocardial infarction within 6 h of chest pain. When used as a lone marker H-FABP has no additional diagnostic value than the troponin I which is considered as the single best cardiac marker currently. H-FABP deserves further investigation as it may have a role along with troponin I in multimarker approach for the early diagnosis of AMI.

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Compliance with Ethical Standards

Conflict of interest Dr Anand K. Pyati, Dr Basavaraj B. Devaranavadagi, Dr Sanjeev L. Sajjannar, Dr Shashikant V. Nikam, Dr Mohd Shannawaz and Dr Satish Patil declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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CERTIFICATE OF PARTICIPATION

AMBICON 2014

XXII Annual National Conference of
Association of Medical Biochemists of India
Sri Venkateswara Institute of Medical Sciences, Tirupati
(A university established by an act of Andhra Pradesh Legislature)
14th - 16th November 2014

This is to certify that Dr ANAND PYATI has participated in the conference as delegate / faculty. He / She has participated in PG Quiz / PG debate / Faculty debate / Chaired a Session / Delivered an Oration / Lecture / Presented a paper (Platform / Poster) tilted, "Pyati A, Rathi DB, Sajjannar S, Devaranavadagi BB. Diagnostic value of heart-type fatty acid binding protein in the early diagnosis of myocardial infarction."

Dr E. Ravindra Reddy Chairman AP Medical Council	**K. Satyanarayana Murthy** Registrar AP Medical Council	**Dr Jasbinder Kaur** President AMBI	**Dr P.V.L.N. Srinivasa Rao** Organizing Secretary AMBICON- 2014	**Dr B. Vengamma** Director cum Vice Chancellor SVIMS University

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