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CURRENT BIOMARKERS FOR MYOCARDIAL INFARCTION

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ABSTRACT

Myocardial infarction (MI) is a medical condition that occurs, when a coronary artery is severely blocked by vulnerable plaque and as a result, there is a significant reduction or break in the blood supply leading to cardiac damage. Cardiac biomarkers play a major role in the investigation of MI. The current study focused on the lipid profile patterns and cardiac biomarker levels in MI patients and in MI prone subjects. The study also discusses on the role and sensitivity of Troponin I over other biomarkers. The study was carried out in three groups viz., normal subjects, MI prone subjects and MI patients. Lipid profile and cardiac biomarkers like Troponin I, CK, CKMB, SGOT were analyzed in all three groups and compared. Increased levels of Total cholesterol, Triglyceride, Low density lipoprotein (LDL), Very low density lipoprotein (VLDL) were observed in both MI patients and MI prone category ($P<0.001$), whereas the level of High density lipoprotein (HDL) was decreased in both the study groups compared to the normal control. Elevated levels of Troponin I, Creatine Kinase(CK), CK-MB and Aspartate transaminase (AST/SGOT) were noticed in MI patients relative to other groups. MI prone subjects were found to have discordant biomarker levels in their blood with normal Troponin I, SGOT levels and elevated CK, CKMB levels. Troponin I is specific and sensitive to cardiac damage and hence can be used in the diagnosis of MI and early detection of cardiovascular risk. Subjects prone to CVD can be found out by their lipid profile patterns and discordant biomarker levels.

KEY WORDS: **Troponin I, biomarkers, Myocardial infarction, lipid profile, cardiac damage**

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INTRODUCTION

Ischemic heart disease is the leading cause of death in developed countries, but third to AIDS and lower respiratory infections in developing countries [1]. Myocardial infarction, more commonly known as heart attack, is a common presentation of ischemic heart disease or coronary artery disease. It is a medical condition that occurs, when a coronary artery is severely blocked by vulnerable plaque and as a result of that there is a significant reduction or break in the blood supply. The patient may experience significant disability or die as a result of MI, depending on the extent of myocardial damage. Myocardial Infarction may result from a coronary artery spasm which can occur in both normal blood vessels and those partially blocked by plaques and the cause of coronary artery spasm is still unknown. In India, cardiovascular disease is the leading cause of death [2]. The deaths due to cardiovascular diseases(CVD) in India were 32% of all deaths in 2007 and are expected to rise from 1.17 million in 1990 and 1.59 million in 2000 to 2.03 million in 2010 [3,4]. It is predicted that the mortality in Indians due to cardiovascular diseases would rise by 103% in men and 90% in women between 1985 and 2015 [5]. The risk factors associated with a higher incidence of MI can be classified into modifiable and non – modifiable risk factors. Modifiable risk factors are smoking, hypertension, hyperlipidemia, obesity, diabetes mellitus, lack of physical activity, stress and hyperhomocysteinemia whereas, Non – modifiable risk factors include, age, gender, family history or race [6, 7]. Other factors like hypothalamic pituitary disease [8], chronic renal failure in diabetic patients[9] also accounts to higher incidence of MI. The pathophysiology includes atherogenesis [10] and plaque rupture [11, 12, 13]. The diagnosis of myocardial infarction is made by integrating the history of the presenting illness and physical examination with ECG findings and cardiac markers (blood tests for heart muscle cell damage). A chest radiograph and routine blood tests may indicate complications or precipitating causes and are often performed upon arrival to an emergency department. New regional wall motion abnormalities on an echocardiogram are also suggestive of myocardial infarction [14]. WHO criteria [15, 16] have classically been used to diagnose MI. A patient is diagnosed with myocardial infarction if two (probable) or three (definite) of the following criteria are satisfied: 1) Clinical history of ischaemic type chest pain lasting for more than 20 minutes. 2) Changes in serial ECG tracings 3) Rise and fall of serum cardiac biomarkers such as creatine kinase-MB fraction and troponin I. According to the new guidelines, a rise in cardiac troponin level accompanied by either typical symptom, pathological Q waves, ST elevation or depression or coronary intervention are diagnostic of MI. Other nonspecific findings include fever, leucocytosis, erythrocyte sedimentation rate and C - reactive protein in acute phase allow quantification of myocardial infarction extension [17].

Cardiac markers are substances released from heart muscle when it is damaged as a result of myocardial infarction. Intracellular proteins, modified by disease, may be released from the myocardium and detected in patient's blood. Currently used diagnostics for myocardial injury are based on the detection of such intracellular proteins, including cardiac troponin I and T (cTnI and cTnT), myoglobin, creatine kinase (CK), the MB isoenzyme of CK (CKMB), aspartate transaminase (AST) and lactate dehydrogenase. Cardiac troponins are considered as the new laboratory standard for myocardial infarction diagnosis as well as for diagnosis and management of unstable angina, because of it's, superior tissue specificity and prolonged time window of elevation. Unlike the other cardiac biomarkers, however, the troponins provide additional information about the functional consequences of infarction [18]. Troponin lies

within the groove between actin filaments and is attached to the protein tropomyosin, in both skeletal and cardiac muscle tissue. Troponin has three subunits, TnC, TnI, and TnT, Cardiac troponin I and T are very sensitive and specific indicators of damage to the heart muscle (myocardium). Creatine kinase (CK) is an enzyme (EC 2.7.3.2) expressed by various tissue types. It is also called as Phosphocreatine kinase or Creatine PhosphoKinase (CPK). It consists of 2 subunits, B – brain type, M – muscle type and hence there are three different isoenzymes CKMM – muscle, CKBB – brain, CKMB – myocardium. All these isoenzymes are expressed at different levels in different tissues. In acute myocardial infarction, plasma CKMB raises 4-6 hours after the onset of chest pain, peaks within 12-24 hours, and returns to baseline levels within 24-48 hours. One CKMB measurement, even when taken at an appropriate time, cannot definitively confirm or rule out the occurrence of AMI, hence serial CKMB determinations is more informative than a single determination. [19]. Aspartate transaminase (AST) also called serum glutamate oxaloacetate transaminase (SGOT) or aspartate aminotransferase (EC 2.6.1.1), was defined as a biochemical marker for the diagnosis of acute myocardial infarction in 1954. However the use of AST for such a diagnosis is now redundant and has been superseded by the cardiac troponins [20].

Troponin along with other cardiac biomarkers may provide complimentary information on the underlying pathobiology and prognosis in an individual patient. The analytic sensitivity for myocardial damage may increase with Troponin as biomarker and may offer insights in the timing and mechanism of myocardial injury [21, 22]. Other inflammatory markers currently under investigation include C-reactive protein, lipoprotein associated phospholipase A_2, myeloperoxidase and pregnancy associated plasma protein A. Increase in blood cholesterol level has a greater risk of developing heart disease or atherosclerosis [23]. Lipoproteins are a biochemical assembly that contains both proteins and lipids and they carry fats and cholesterol around the body. The interaction of these lipoproteins with enzymes in the blood, with specific proteins on the surfaces of cells determine whether triglycerides and cholesterol will be added to or removed from the lipoprotein transport particles. Regarding atheroma development and progression vs. regression, the key issue has always been cholesterol transport patterns, not cholesterol concentration itself. The aim of the present study was to study the lipid profile patterns and the cardiac biomarkers level in MI patients and also in MI prone subjects. The current study had also focussed on the association of cardiac markers and the lipid profile patterns with the risk of MI.

MATERIALS AND METHODS

Study subjects

This prospective study was done on 25 patients (group A) who were admitted in Vadamalayan Hospitals, Madurai. They were subjected to ECG and other clinical examinations to confirm MI. Another 25 patients were selected based on the symptoms and they were categorized as group B (MI prone). The control group consists of 25 age and sex matched healthy volunteers. Blood from patients with myocardial infarction were drawn at the time of admission whereas; from the myocardial infarction prone category and normal controls, the blood was drawn between 6 -7 am (fasting sample) by venipuncture. The collected blood was allowed to clot at room temperature (30 - 35°C) after which the serum was separated by centrifugation. All the analysis was carried out on the same day of collection of samples.

Biochemical Estimation

Total cholesterol was estimated by Richmond method [24], serum TG was estimated by GPO – PAP method (enzymatic method using glyceraldehydes 3 phosphate oxidase). Serum HDL was estimated by precipitation method

(precipitation of VLDL and LDL by Mg ions and phosphotungstic acid followed by estimation of HDL by cholesterol esterase). Serum LDL was quantified by calculation using a formula (total cholesterol – (TG/5 + HDL)). Serum VLDL was estimated by calculation using a formula (TG/5). The activity level of CKMB was determined by Morin method [25]. Serum Creatine kinase was quantified using UV kinetic method. Serum SGOT was assayed by following the Expert panel of the IFCC on enzymes, 1976[26]. Serum Cardiac Troponin I was determined by one step immuno chromatography assay method.

Statistical analysis

The values obtained were expressed as mean ± SD. Statistical analysis was done by students't' test and 'p' value was arrived at to assess the statistical significance of changes observed. Values less than 0.02 were considered significant.

RESULTS AND DISCUSSION

Table 1 shows the lipid profile of MI patients, MI prone subjects and normal healthy controls. From the results it was observed that the levels of TG, LDL, VLDL, total cholesterol were elevated in MI patients than in normal controls. The levels of these lipids were also increased in MI prone subjects. HDL, the good cholesterol was found to be lesser in both MI prone subjects and in MI patients. These findings were in accordance with that of Sarkar *et al*., [27] who have stated that the serum total cholesterol, triglyceride, LDL and VLDL levels were higher in hypertensive patients and HDL levels were lower in them. In the present study almost all patients with myocardial infarction were hypertensive. Aghaeishahsavari *et al*., [28] have reported that the increased serum levels of triglyceride, total cholesterol, and LDL were observed in obese patients who were confirmed with CVD. They also had low levels of HDL than their age-matched persons having normal weight. High cholesterol levels were associated with heart disease. The high levels of cholesterol and triglyceride in most people were a result of increased consumption of foods rich in fat, smoking, lack of exercise, disease states that affect lipid metabolism like diabetes, hypertension, hypothyroidism, obesity, other hormonal imbalances like growth hormone deficiency, liver and kidney diseases and alcoholism. Low levels of HDL cholesterol are also associated with increased risk of heart disease [29]. Decrease in HDL level is a serious indication of development of cardiovascular disease, because HDL has the capacity to scavenge cholesterol from extra hepatic tissues and from artery lumen to liver for re-utilization. LDL at high concentrations invades the endothelium of arteries and become oxidized. This oxidized LDL triggers the formation of atheromas. VLDL level accelerates the rate of formation of atheromas. Bachorik *et al*., [30] have reported that HDL cholesterol levels are positively associated with physical activity i.e. the more physically active, the higher the HDL. It is also inversely associated with body weight: the more overweight, the lower the HDL. Low HDL level confers an increase in cardiovascular risk. Lipoproteins and especially the cholesterol predict the risk of cardiovascular disease. Table2 shows the activity levels of CK and CKMB in MI patients, MI prone subjects and in normal controls. From the studies it was noted that the activity level of creatine kinase (total) was elevated more than three folds than the normal range whereas the creatine kinase –MB isoform was found to be elevated more than 15 folds in MI patients. It was also clear that these enzyme levels were also increased in MI prone subjects. Phosphocreatine serves as an energy reservoir for the rapid regeneration of ATP in tissues that consume ATP rapidly, especially skeletal muscle, brain and smooth muscle. Clinically, creatine kinase is assayed in blood tests as a marker of myocardial infarction, rhabdomyolysis and in acute renal failure. Elevation of CK is an indication of damage to muscle - it may be injury, myocardial infarction, rhabdomyolysis, etc., Lowered CK can be an indication of alcoholic

liver disease and rheumatoid arthritis. CK-MB is one of the most important myocardial markers, with well-established roles in confirming acute myocardial infarction (AMI) and in monitoring reperfusion during thrombolytic therapy following AMI. Romic *et al*., [31] have reported that CKMB is sensitive and is released into circulation within 2 to 4 hrs the onset of MI. McLauchlan [32] have stated that total CK activity shows an increase following myocardial infarction in which it is increased earlier than other enzymes. Since CK, SGOT were not peculiar to myocardial tissue, raised CKMB levels were most useful. If the total activity was raised and CKMB contributes more than 6% of the activity, then myocardial infarction is considered highly probable. Adams *et al*., [19] have found out that high level of CK, CKMB might reflect skeletal injury rather than myocardial damage. But in the present study none of them had any muscular damage hence the increase in CK and CKMB may be due to cardiac problem.

Table 3 depicts the level of Cardiac Troponin I and SGOT in myocardial patients, Mi prone subjects and in normal controls. The SGOT activity level was increased to a very greater extent than the normal and the cardiac troponin I level was elevated to eighty times than the normal. Troponin I was only found in the myocardium in adults, making it extremely specific for cardiac disease. It is also found in much higher concentration than CKMB in cardiac muscle. Troponin I is not found in detectable amounts in the serum of normal individuals, patients with multiple injuries, in athletes after strenuous exercise or in patients with elevated CKMB unless myocardial infarction is present. The major disadvantages of CKMB are the lack of heart specificity, presence of small amounts in normal myocardium tissue and detectable levels in normal individuals as they are released into sera as a part of normal clearance mechanisms [33]. Robert Dufour [34] have found out that Cardiac troponin I was found to be increased in myocardial ischemia in an experimental animal model, even in the absence of morphologic or ultrastructural evidence of cell death. Thus troponin I can be used for the recognition of risk in patients with an acute coronary syndrome. Scirica and Morrow [35] have pointed out that even low level elevation of troponin I correlate with higher risk of death and recurrent ischemic events compared with levels of troponin below the decision limit. They have further noted that cardiac troponin is extremely specific for myocardial damage. According to Valgimigli *et al*., [21] measurement of circulating levels of troponin has proven to be sensitive and specific test for cardiac damage detection. From table 3, it was significant that though SGOT, the famous cardiac marker was increased to five folds on MI onset, the cardiac troponin I was found to be elevated for more than 70 folds. Early diagnosis of acute myocardial infarction and risk stratification is possible with the use of a sensitive assay for troponin I, regardless of the time of chest-pain onset [36]. The diagnosis of MI and identification of patients at high risk of recurrent MI and death is increased with the implementation of a sensitive troponin assay [37]. Thus, from the present study it can be derived that cardiac troponin can be considered as a better biomarker for myocardial infarction than the SGOT. From Table 2 & 3, it is evident that the MI prone patient group has discordant cardiac biomarkers with elevated CKMB levels and normal Troponin I. This is in accordance with the findings of Storrow *et al.,* [38], an increased CKMB level regardless of troponin level identify patients at higher risk for acute coronary syndrome than those with uniformly normal cardiac biomarker levels. Discordant cardiac biomarkers may identify patients at increased risk for acute coronary syndrome. Myocardial infarction prone category should concentrate more on lowering their lipid level. Cholesterol lowering medicines and serious life style modifications are advised to avoid myocardial infarction. Early diagnosis of myocardial infarction is not possible because early and sensitive markers for myocardial damage have not yet identified.

The present cardiac markers also have some disadvantages. Hence, more study on early biomarkers is required. Some of the early markers under consideration are C – reactive protein, lipoprotein associated phospholipase A_2, myeloperoxidase, Heart-type Fatty Acid Binding Protein, N-Terminal Pro-B-type Natriuretic Peptide and copeptin etc.

Table 1
Lipid profile

CATEGORY	TOTAL CHOLESTEROL (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
NORMAL CONTROL	170.44 ± 20.36	81.6 ± 15.45	56.92 ± 9.97	86.84 ± 8.37	19.12 ± 6.74
MI PRONE SUBJECTS	238 ± 31.53	202.28 ± 41.34	34 ± 10.47	138.12 ± 44.17	45.32 ± 14.10
MI PATIENTS	237.76 ± 31.37	237.44 ± 105.35	39.29 ± 5.46	144.76 ± 33.56	49.8 ± 15.38
Significance	a) @ b) @ c) €	a) @ b) @ c) $	a) @ b) @ c) ¥	a) @ b) @ c) €	a) @ b) @ c) €

@ P<0.001 – highly significant, $ P<0.01 – moderate significant, ¥ P<0.05 – Less significant, € – non significant
a) MI patients Vs Normal controls
b) MI prone subjects Vs Normal subjects
c) MI prone subjects Vs MI patients

Table 2
Activity levels of CK & CKMB Isoform

CATEGORY	CK (U/L)	CKMB (U/L)
NORMAL CONTROLS	64.96 ± 24.49	8.08 ± 4.23
MI PRONE SUBJECTS	90.8 ± 23.42	13.36 ± 5.41
MI PATIENTS	444.84 ± 223.22	120.52 ± 101.98
SIGNIFICANCE	a) @ b) @ c) @	a) @ b) @ c) @

@ P<0.001 – highly significant
a) MI patients Vs Normal controls
b) MI prone subjects Vs Normal subjects
c) MI prone subjects Vs MI patients

Table 3
Level of Cardiac Troponin I and SGOT

CATEGORY	CARDIAC TROPONIN I (ng/dl)	SGOT U/L
NORMAL CONTROLS	0.5724 ± 0.23	24.88 ± 5.84
MI PRONE SUBJECTS	0.5936 ± 0.21	26.96 ± 10.76
MI PATIENTS	40.25 ± 37.48	135.92 ± 98.38
SIGNIFICANCE	a) @ b) € c) @	a) @ b) € c) @

@ P<0.001 – highly significant, € – non significant,
a) MI patients Vs Normal controls
b) MI prone subjects Vs Normal subjects
c) MI prone subjects Vs MI patients

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