A Comparative Study of Troponin T as an Innovative Biomarker with Gold Standard Parameters in Acute Myocardial Infarction.

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ABSTRACT :

Background : Myocardial Infarction (MI) is the leading cause of the death in the developed world. The biomarkers have an essential role in the diagnosis, guiding management and clinical decision making in the setting of patients presenting with signs and symptoms of myocardial infarction. **Aim :** The goal of study was to scrutinize collision of all the baseline parameters, serum troponin T as an innovative biomarker in comparison with CK and CK-MB in acute myocardial infarction patients. **Objectives :** The intention of study was to inspect impact of serum Troponin T, CK, CK-MB along with lipid profile as baseline parameters in patients with acute myocardial infarction and compared with the healthy controls. **Study Design :** In present case control study, 45 myocardial infarction patients were estimated and compared with 45 control subjects. The concentration of cTnT was measured by immunoassay method. Estimation of serum CK-MB and CK by Liquid stable optimized UV method/ immunoinhibition method. **Result :** A significant elevation were found in the levels of serum troponin T, CK and CK-MB ($p<0.001$) in study group patients when compared with healthy controls. The cardiac troponin T has been shown to be highly sensitive for cardiac injury and not elevated in any other trauma or skeletal muscle injury. Cardiac troponin T is ordinarily undetectable in healthy individuals, and hence its measurement can serve as a innovative tool in diagnosis of AMI.

Key Words : Acute myocardial infarction, (AMI), Troponin T, CK, CK-MB.

Introduction : Acute myocardial infarction (AMI) is the leading cause in both developed and developing countries. Despite impressive strides in diagnosis and management over the past three decades, AMI continues to be a major public health problem in the industrialized world and is becoming an increasingly important problem in developing countries [1].

Over the past 50 years, it has become clear that the cascade of thrombotic events following atherosclerotic plaque rupture causes occlusion of coronary artery, interrupting blood supply and oxygen to myocardium, and this result in infarction[2]. MI is the cause of 25-30% of deaths in most industrialized countries. The WHO has drawn attention to the fact that AMI is our modern epidemic. There is large data exist in the hospitalized patients regarding the occurrence of AMI[3]. However, there are only two studies on its prevalence in the general population of India. The prevalence was found to be 65.4 and 47.8 per 1000 males and females respectively in urban population[4] 22.8 and 17.8 per 1000 males and females respectively in rural population[5].

MI results from the thrombotic cascade events followed by atherosclerotic cascade events leads to the atherosclerotic plaque rupture. This causes occlusion of the coronary artery mainly affects interrupting the blood supply and oxygen to myocardium. This all events leads towards the MI[2,6].

The diagnosis of AMI is based on clinical symptoms, Electrocardiographic (ECG) findings, characteristic pattern of changes in some serum enzymes such as CK-MB, lactate dehydrogenase and its isoenzymes (LDH), aspartate transaminase (AST), cardiac troponins like troponin I, troponin T etc[7]. ECG is commonly used method of choice for diagnosis of MI but maximum times ECG shows inconclusive patterns [8] and hence the estimation of various important serum biochemical markers of myocardial injury arises to confirm the diagnosis of myocardial injury.

As per the refined WHO criteria 2000, it gives more prominence to cardiac biomarkers[9]. According to new guidelines, a cardiac troponin rise accompanied Q waves, ST- Elevation or depression or coronary intervention is diagnostic criteria of MI [9].

A new immunoassay of cardiac troponin T (cTnT) which eliminates most of the earlier shortcomings of laboratory infarction diagnostics was planned it was

carried out to evaluate the utility values of troponin T in early diagnosis of AMI. Elevated levels of Troponin T, Troponin I, CK-MB have been regarded as innovative biochemical markers of myocyte necrosis. CK, CK-MB still have a formal place in defining MI. These enzymes normally exits in cellular compartment and leak out into the plasma during myocardial injury due to disintegration of contractile elements and sarcoplasmic reticulum. Troponin T has high myocardial tissue specificity and offers an improved sensitivity and specificity for MI compared to traditional biochemical markers.

Material and Methods :

The present study was carried out in the Department of Biochemistry, Mahatma Gandhi Memorial College and Hospital, Warangal (A.P.) and Department of Biochemistry, PDVVPF, s Medical College, Ahmednagar, Maharashtra. Prior to start the study, local institutional ethical clearance was obtained and utmost care was taken during experimental procedure according to the Declaration of Helsinki 1964.

Study type : Analytical case-control study.

Protocol :

Control group : This study has been performed on total 90 subjects which includes 45 age and sex matched (31 males and 14 females) healthy controls which mostly staffs and PG students in the age group 30-60 years. All they are absolutely healthy and excluded having risk of cardiac catheterization, coronary artery disease, aortic dissection, pulmonary embolism, diabetes mellitus, hypertension etc.

Study group : it includes 45 (32 males and 13 females) patients from the age group 30-60 years. All the patients were admitted in the Mahatma Gandhi Memorial college and Hospital, Warangal after the onset of chest pain showing ECG changes suggestive of AMI, ST segment elevation >0.2 mV presenting within 6 hours from chest pain onset were included in the study.

After obtaining a written consent from all the subjects who were included in the study and by giving detail information of study, blood samples were collected from controls and patients. Total 5 ml blood was withdrawn aseptically from the antecubital vein from each subject in a plain container. The samples were centrifuged at 3000 rpm for 10 min to separate serum. Lipaemic and icteric samples were discarded.

The serum total cholesterol and HDL- cholesterol were estimated by using CHOD-PAP fully automated method [10,11]. The concentration of cTnT was measured by immunoassay method [12]. Estimation of serum CK-MB and CK by Liquid stable optimized UV method/ immunoinhibition method [13,14]. The TG by CHOD-PAP end point assay method[15].

Statistical Analysis :

The statistical analysis was carried out by using the SPSS (Statistical Package for Social Sciences) statistical software, version 17.0 for Windows. The Student's 'z' test were applied for the significance and the results were expressed in mean ± SD. p values ($p<0.001$) were considered as highly significant.

The Receiver Operating Curve (ROC) analysis was performed and the area under the curve was determined for the assessment of the diagnostic performance of serum cTnT levels at best cut off value in discriminating control Vs cases on day 1st day of admission after the onset. The optimum cut off values for the determination of serum cTnT in controls and patients were selected from ROC analysis. The optimum cut off value was used for calculating the diagnostic sensitivity and specificity of cTnT on day 1st AMI.

Results : A total of 90 patients were screened for the study. After applying inclusion and exclusion criteria, total of 45 patients were eligible for the study. Most of the patients were in the age group 30-60 years and were male 75%. The number of patients belonging to the rular areas (75%) was farmers and tobacco chewers.

Table-1: Shows the baseline demographic clinical characterization of patients and the healthy control groups.

Sr. No	Variables	Controls (n=45) Mean±SD	Patients (n=45) Mean±SD	P value <
1.	Age in years	39.8±20.13	47.4±12.01	0.001*
2.	Gender male/female	31/14	32/13	0.001*
3.	Total cholesterol (mg/dl)	179.73±19.23	268.13±82.87	0.001*
4.	HDL (mg/dl)	68.67±4.21	35.87±6.95	0.001*
5.	LDL (mg/dl)	80.63±17.5	169.4±29.7	0.001*
6.	VLDL (mg/dl)	23.56±10.5	60.98±17.5	0.001*
7.	TG (mg/dl)	131.53±11.45	280.68±77.1	0.001*

Table -1 shows the baseline characteristics of all the subjects.

Values were expressed in mean with standard deviation (Mean±SD).

*indicates statistically high significant (p<0.001)

n=number of patients.

Table - 2 shows the comparison of serum cTnT level, CK, CK-MB within 6 hours of the patient's admission and controls.

Sr. No	Variables	Controls (n=45) Mean±SD	Patients (n=45) Mean±SD	P value <
1.	cTnT (µg/ml)	0.011±0.007	5.14±2.7	0.001*
2.	CK (U/L)	60.5±21.7	258.53±74.06	0.001*
3.	CK-MB (U/L)	7.96±8.07	90.75±73.67	0.001*

The performance of cTnT of control Vs cases on day 1 after the onset of MI has been presented in table -3.

Table -3- Showing the performance of cTcT of control Vs cases on day 1of onset of AMI.

Parameter	Area under curve	Best cut off value	Sensitivity	Specificity	Diagnostic efficiency
cTnT[control Vs cases on day 1st of onset)	0.9967	4013	100%	94.03%	97.67%

The sensitivity, specificity, positive predictive value, and negative predictive value were analyzed. The combined use of cTnT on day 1st and after treatment significantly improves the sensitivity 100%, specificity 94.03%, and diagnostic efficiency 97.67%. The optimum diagnostic cut off value was 4013. The area under curve for cTnT on day 1st was 0.996.

Discussion : The diagnosis of acute myocardial infarction (AMI) has been traditionally based on the characteristic clinical history, ECG abnormalities, and increased serum concentrations of cardiac marker enzymes like cardiac troponin T (cTnT), CK, CK-MB, LDH, and AST etc. [16,17]. Comparison of cTnT, CK, CK-MB, lipid profile originating exclusively from the myocardium. The measurement of these serum enzymes as a reflection of damage to myocardial muscle cells plays an important role in the diagnosis of AMI [18].

Cardiac troponin T is cardio specific, highly sensitive marker for myocardial damage. The value is detected from 3 hours upto 5 days and remains high till 14 days whereas CK, CK-MB elevation typically begins from 4-6 hrs after onset of infarction and is not high till 20 hours after onset of occlusion[17,18].

CK-MB rises in number of conditions such as aortic stenosis hypertrophied myocardium, malignancies, inflammatory myopathies, collagen vascular diseases, scleroderma etc. and due to this reason it loses specificity of the investigation of CK, CK-MB test [19].

In our study after admission of patient the CK and CK-MB estimation were carried out at 6 hours and both parameters were significantly enhanced (p<0.001) when compared with the healthy controls which supported by the other researchers [20,21].

The cardiac troponins like troponin T and troponin I are well established biomarkers for diagnosis and prognosis of AMI. The measurement of both these parameters that is troponin T and troponin I are now a crucial step in new diagnostic criteria for MI[22].

In the present study extremely elevated concentration of cTnT was found (p<0.001) when compared with control group. Our data was strongly supported by the scientist Ooi DS et al. As per their research the possible mechanism to increase the cTnT may be due to endothelial dysfunction, acute cardiac stretch, microinfarction and left ventricular hypertrophy [23]. The troponin T is a part of the regulatory system of contractile complex of skeletal and heart muscle. After loss of the integrity of the cell membrane, it is released into the circulation similar to myoglobin or creatinine kinase. As the cardiac troponin T is ordinarily undetectable in healthy individuals 7 hence its measurement in serum is a powerful tool in the diagnosis of AMI.

After the myocardial cell necrosis cardiac troponin T concentration is immediately increased and remains as for one week [24]. Thus cardiac troponin T measurement is particularly valuable in clinical circumstances in which traditional enzyme determination fail to diagnose myocardial cell damage efficiently.

According to Katus et al, troponin T is elevated more than 20 times of the analytical sensitivity of the assay in all patients with infarction. Troponin T appeared in the serum as early as 3 hours after the onset of chest pain in 50% of patients and remained elevated in all patients for more than 130 hours [17].

Conclusion : The essence of the current study lies in the fact that, there are twenty times higher concentration of cardiac troponin T than the normal blood donors in AMI patients at the time of admission. cTnT in serum appears to be a more sensitive and

innovative indicator of myocardial cell injury than CK and CK-MB activity. Hence detection of cTnT in the circulation may be useful as early prognostic marker in patients with AMI.

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