ORIGINAL ARTICLE

Troponin T and Cardiac Enzyme Levels since Onset of Chest Pain in Patients Suspected of Acute Myocardial Infarction (AMI) in Punjab, Pakistan

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ABSTRACT

Objective: To early diagnose the patients of AMI by using cardiac markers like CK, CK-MB, to find out the significance of these enzymes in comparison with high sensitive Troponin T hs and to find out the presence of a micro-infarction by using newly developed cTnT hs method.

Patients and Methods: This was a cross sectional study, conducted at Punjab Institute of Cardiology and included 545 patients. Blood sample of every patient "Suspected of AMI" was sent to the laboratory for estimation of CK, CK-MB by conventional kit method and a high sensitive Troponin T reagent kit was used for prompt diagnosis of AMI. Patients of varying age were included and demographic details like hypertension, diabetes and smoking, family history of IHD, were collected to find out association with AMI.

Results: Out of 545 study participants 62.65% (n=341) were male and 37.43% (n=204) were females, the mean age was 54.8 ± 13.42 years, the most frequent risk factor seen was hypertension (HTN in 72.0 % (n=90), followed by Smoking 45.5% (n=90), Diabetes mellitus (DM) 43.3% (n=90), and frequency of family history was seen 18.8% (n=90) in patients positive for acute myocardial infarction (AMI).

78.8% (n=90) males and 21.2%(n=90) females, indicated more prevalence of AMI in male population with frequency of AMI in patients from 41-60 years of age.

There was significant association between hypertension, smoking and diabetes with AMI. Family history was not significantly associated with the incidence of AMI.

CPK, CK-MB were performed on patient's serum sample according to the procedures determined by reagent kits of Roche Diagnostic company on Chemistry Analyzer Hitachi 912. Optimized UV test method was used according to DGKC (German Society of Clinical Chemistry and Laboratory Medicine) and IFCC.

Special Chemistry analyzer Cobas e 411, automated instrument was used for the quantitative measurement of Troponin T by using Chemiluminescence method (Troponin T hs reagent kits of Roche Diagnostic Company).

Conclusion: It was concluded that a direct relationship exists between Troponin T and CPK, and CK-MB was more specific with even more closer and linear relationship with Troponin T hs**.** It was further observed that high sensitive Troponin T reagent method alone was sufficient to make a final diagnosis of AMI. Troponin T hs alone not only reduces the investigation time but reduces the overall cost as well.

Keywords: Acute Myocardial Infarction.

INTRODUCTION

Majority of the patients coming to the hospital with chest pain at the emergency department, may have a non-specific chest pain but it is the most important reason for taking urgent medical care of such patients from which few of these may have acute coronary syndrome. Such patients are therefore investigated for myocardial infarction (MI). Blood samples sent to diagnostic laboratory are tested for cardiac markers in serum in the early hours after the onset of symptoms, not to detect acute myocardial infarction (AMI) but with the aim to exclude MI. Early ruling out of AMI will result into the proper management of these patients, as patients with a low probability of coronary artery disease if admitted will not only increase the cost of the hospitals but will leave a little space for actual patients suffering from myocardial infarction [1].

On the other hand, some patients may not be diagnosed timely and this delay may result in delayed administration of treatment or even no administration. Some of such patients may be discharged from emergency department of the hospital leading to serious illness and even legal implications. It becomes mandatory therefore, to diagnose and differentiate, into the patients with myocardial infarction (MI) and patients coming to emergency department with chest pain.

According to old criteria set by World Health Organization, the diagnosis of AMI requires the presence of at least two of the following markers: (**a**) history of typical chest pain, (**b**) evolutionary changes of the electrocardiogram (ECG), (**c**) rise in the level of cardiac biomarkers in serial serum samples [2.] Old definition has been replaced with a latest definition of myocardial infarction by The joint European Society of Cardiology and American college of Cardiology (ESC/ACC) Committee, which redefined the Acute Myocardial Infarction (AMI) with reference to cardiac biomarkers as mentioned here: 1) an elevated value of cardiac Troponin "cTnI" or "cTnT" which exceeds the decision limit of 99th percentile of the value for a reference control group, on at least one occasion; 2) an increased value of creatine phosphokinase (CPK) and isoenzyme of creatine phosphokinase (CK MB) 99th percentile of the value for a reference group on at least two different "test results" with a typical pattern of rise and fall or a value of greater than or twice the upper limit of the reference range at any one sample.[3]

Biomarkers with increased degree of specificity and sensitivity have been developed for the diagnosis of AMI but none of these singly fulfill the criteria for final diagnosis. An ideal biomarker would be one with high clinical sensitivity and specificity, appearing early in circulating blood after AMI, facilitating early diagnosis, remaining abnormal or elevated for several days, after Acute Myocardial Infarction and could be assayed with a rapid turn-out time. so that results could be obtained fast enough to influence the decision-making process regarding patients' triage and management[4]. The chest pain "Starting time"is also very important clue in the diagnosis of AMI but different patients coming to emergency department may have great variation in this regard.[5]

Therefore different markers which are very much helpful, with short turnout time including blood markers such as cardiac

enzymes like Creatine phosphokinase(CPK), CK-MB and Troponin T hs are performed, which may be found elevated and are detectable in early myocardial infarction and therefore are of much importance in ruling out the AMI.[6]

Automated quantitative measurement of the above said parameters particularly CPK, CK-MB and Troponin levels are currently the best markers for the definitive diagnosis of AMI. The early appearance of a marker released into the bloodstream soon after an injury may facilitate early diagnosis [7].

Creatine Kinase has clinical sensitivity of almost 90 % for the diagnosis of AMI but unfortunately it is not highly specific as absolute concentrations of total CK may be found increased in some muscular disease or due to some procedure like angioplasty or cardiac surgery, even CK-MB values may also be found raised to some extent [8]. CK-MB is heart specific and is considered an ideal biomarker for the final diagnosis of acute myocardial infarction (AMI).[9]

Activity of Serum total Creatine Kinase (CK) and CK-MB "concentration" rise in parallel following myocardial injury, starting to increase 4±6 h after injury, reaching peak serum concentrations after 12±24 h and returns to baseline, after 48±72 h [10].

Release kinetics of CK and CK-MB are same, whereas CK-MB has sensitivity and specificity of more than 90% for the diagnosis of AMI but this sensitivity and specificity changes with the time of presentation and after the onset of the symptoms. [11].

The availability of a more sensitive, specific biomarker with a short turnaround time is more helpful in the diagnosis of AMI and therefore for the management of the patient. Troponin is one of such marker used now a day's for early detection of AMI [12].

Troponin I (cTnI i.e. cardiac Troponin I) and Troponin T (cTnT i.e. cardiac Troponin T) are two regulatory proteins present in cardiac muscle which control the calcium mediated interactions between actin and myosin of heart tissue [13].

The use of serum cTnI and cTnT is very specific, sensitive and currently the best marker, for the definitive diagnosis of AMI and have a crucial role in the assessment of damage to cardiac tissue, particularly in combination with cardiac enzymes. Elevated values of cardiac enzymes and Troponin levels are now used as a "standard biomarker" for the final diagnosis of coronary artery disease or heart attack[14].

Troponin Tand Troponin I, both begin to rise in 4–12 hours after ischemic heart injury and reaches at peak level after 12–24 hours and remain abnormal for 4-10 days. Results are not increased in the presence of skeletal muscle Troponin[15].

Cardiac troponins are particularly compared with CK-MB for the diagnosis of Acute myocardial injury (AMI) . Troponin T is also present in small quantities in skeletal muscles but methods currently available are highly sensitive with improved analytical efficacy and specificity to measure cardiac Troponin only and do not measure the muscle Troponin T and is known as cardiac Troponin T high sensitive (cTnT hs) reagent kit. [16] Cardiac Troponins are detected in the serum by using monoclonal antibodies to epitopes of cTnI and cTnT. These antibodies are highly specific for cardiac troponin and have very negligible cross-reactivity with skeletal muscle troponins.[17] This high sensitive assay reagent can detect 10 -100 fold lower limit of cardiac troponin [18] and troponin can be detected even in healthy persons.[19] This highly specific kit has also made it possible to fulfill the requirement for a particular "definition" of acute coronary syndrome (ACS) according to guidelines issued by the National Academy of Clinical Biochemistry in 2007, which stated that in the presence of clinical history of ACS, concentration of Troponin T with a value more than 99th percentile of the levels obtained from a control group at least once, within the 24 hours of the onset of symptoms, is suggestive of acute myocardial infarction.[20]

In an acute coronary condition, troponin levels may not be detectable in patient's serum until 4 hours after the onset of chest pain. It indicates that the patients are at low risk of myocardial damage and should be kept under observation and test should be performed after 6 to 12 hours, to rule out the myocardial infarction[21]. But it is very important to understand that an increased Troponin value is not always due to the myocardial infarction as elevated levels of Troponin will indicate the injury to the myocardium, but does not explain the mechanism of injury and damage to myocardium which may be due to a variety of different other causes of ischemia like pericarditis, pulmonary embolism, acute heart failure, septic shock and as a result of cardio toxic drugs as well as after therapeutic procedures such as coronary angioplasty.[22]

Patients coming in hospital emergency, with pericarditis and pulmonary embolism are the

most important which have been found with abnormally raised levels of troponin along with raised values of CK-MB [18,19,20]. Another reason for increased blood level of troponin in renal failure is its decreased clearance by the kidneys.[23,24].

A complete history of patient including the onset of chest pain will be more helpful in making the final decision in this regard. The knowledge of the other causes that can raise the level of Troponin will not only help in the diagnosis of the patient but will also help to manage the patient accordingly.

It has been established uptil now that the level of Troponin T is indicative of the severity of myocardial damage from mild to severe and also represents acute or chronic disease as values obtained in chronic conditions remain persistent. It can be finally said therefore that larger the value of cTnT hs, higher the probability of AMI and a repeat measurement of Troponin T after 4-6 hours will be much more helpful in differentiation of acute or chronic MI[25].

MATERIALS AND METHODS

Sampling: Patients having chest pain coming to the emergency department of Punjab Institute of Cardiology ranging from 18 to 80 years of age were included in this study for comparison of Troponin T with cardiac enzymes, particularly CK and CK-MB were performed on these patients to rule-out AMI. All patients were informed that their sample will be used to perform tests to confirm or rule-out acute myocardial infarction. Total 545 samples were collected and 341 were males and 204 were females of varying age.

Procedure for Blood Sampling: Blood samples from all patients were collected by using a disposable BD syringe. The blood was allowed to clot. The sample was then transferred into glass tubes and was centrifuged at 3000 rpm for 5 minutes. Clear serum was taken out and was poured in a separate 5 ml tube.

Study Design: This was a cross sectional study on population / participants having chest pain, visiting Punjab institute of cardiology, Lahore were included in this study to rule-out AMI by the performance and comparison of troponin T and cardiac enzymes.

Performance of Tests: CPK, CK-MB were performed on patient's serum sample according to the procedures determined by reagent kits of Roche Diagnostic company on Chemistry Analyzer Hitachi 912. Optimized UV test method was used according to DGKC (German Society of Clinical Chemistry and Laboratory Medicine) and IFCC.

RESULTS

Correlation between cardiac enzymes and Troponin in patients with chest pain was studied by performing the determination of CK, CK-MB and Troponin T hs. Three groups i.e negative, positive and "Suspected of AMI" were made on the basis of reference range of enzymes and Troponin T hs respectively.

Total of 545 subjects coming to hospital emergency, with chest pain were included in this study. Of the 545 patients, 341 were males and 204 females of varying age. The anthropometric data was collected from all subjects, at the time of sample collection. The demographic data included the age, gender, Onset time of chest pain in hours, family history, smoker or non smoker,

had already known ischemic heart disease or not and hypertensive or not .
Statistical Analysis

Table 1: Frequency distribution of population with respect to Gender and percentage .

Gender	Frequency	Percent (%)
Male	341	62.6
Female	204	37.4
Total	545	100

Total 545 subjects were included in this study,who had chest pain and visited Punjab institute of cardiology for further investigations. The population consisted of males 341 (62.60%) and females 204 (37.4 %) .

Table 2: Comparison of miscellaneous demographic variables with Troponin T hs.

Variables		Troponin T hs Groups			Total	p-value
		NEGATIVE for AMI (Less than 14 pg/ml)	SUSPECTED for AMI (from 15 to 99 pg/ml)	POSITIVE for AMI more than 100 pg/ml		
Gender	Male	118	152	71	341	
	Female	86	99	19	204	
Total		204	251	90	545	0.002
Age_group	20 to 40yrs	53	21	11	85	
	41 to 60 yrs	115	134	49	298	
	61 to 80 yrs	36	85	26	147	0.000
	81 to 95 yrs	0	10	4	14	
Total		204	250	90	544	
Hypertension	yes	149	188	65	402	
	no	55	63	25	143	
Total		204	251	90	545	0.846
Diabetic	yes	83	124	39	246	
	no	121	127	51	299	
Total		204	251	90	545	0.166
Smoker	yes	41	56	34	131	
	no	146	180	49	375	
	Ex-smoker	17	15	7	39	
Total		204	251	90	545	0.012
Family History	yes	30	49	17	96	
	no	174	202	73	449	
Total		204	251	90	545	0.383

Fig. 1: Effect of Presence of miscellaneous demographic factors on Troponin T hs
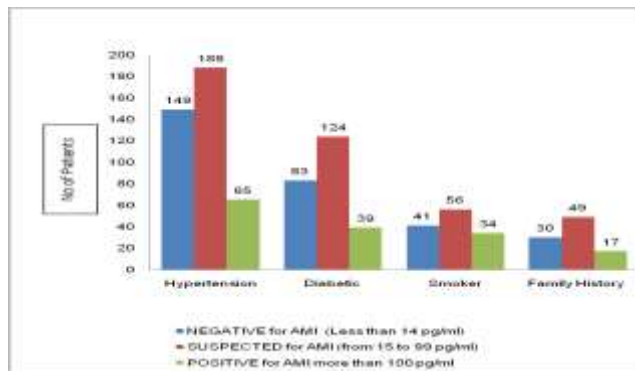
Fig. 2: Comparison of miscellaneous demographic variables present with Troponin T hs.

Effect of absence of miscellaneous demographic factors on Troponin T hs. The demographic data of patients including males and females of varying age ranging from 20 to 85 years But were NOT suffering from Hypertension or Diabetes, were not Smokers and had NO family history of Ischemic heart disease were taken against X-axis whereas number of persons were taken along Y-axis.

Comparison of miscellaneous demographic variables present with Troponin T hs.

Effect of presence of miscellaneous demographic factors on Troponin T hs. The demographic data of patients including males and females of varying age ranging from 20 to 85 years suffering from Hypertension, Diabetes, Smokers and with a family history of Ischemic heart disease was plotted along X-axis whereas number of persons was taken along Y- axis.
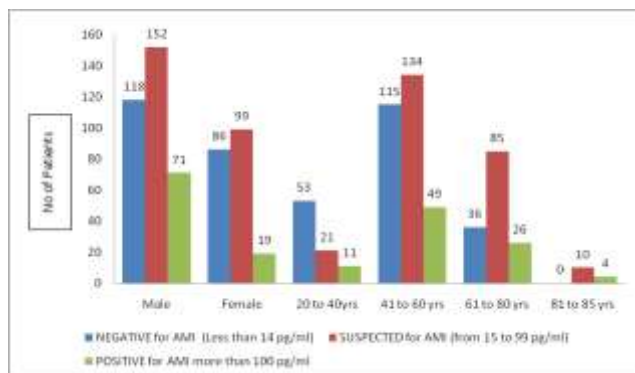
DISCUSSION

This was an Asia specific and gender based particularly local population study. All the patients were investigated for acute myocardial infarction (AMI) by performing Troponin T hs and cardiac enzymes especially CK and CK-MB.

It was observed from the data of patient results that as compared to CK, there was a strong association of CK-MB with Troponin T and in patients of AMI and among local population males were more positive and suspected of AMI than females. Most of the patients having AMI were from 41-60 years of age and visited hospital after 7-12 hours of the chest pain onset.

A large number of local populations suffering from AMI without a family history of ischemic heart disease (IHD) indicated that higher prevalence of AMI may be due to many other contributing factors like hypertension, smoking, diabetes, sedentary life style and younger age.

There were more female diabetics than male diabetics coming to PIC with chest pain. Most of the patients suspected for and positive for AMI on Troponin T basis were more males than

females among the local population and were suffering from hypertension which confirmed the strong association of hypertension with AMI. It was further observed that smoking was the second most common factor for development of AMI.

CONCLUSION

It was concluded that there was a strong association of CK-MB than CK with Troponin T

Therefore it was concluded that CK test being non specific can be avoided from the parameters used to rule-out the AMI. A parallel rise in CK-MB and Troponin T hs therefore indicated that CK-MB can even be skipped or removed from the requisition of emergency tests to reduce the cost and reporting time by the use of high sensitive Troponin T (cTnT hs) alone which was found sufficient to diagnose the possibility of AMI, accurately and relatively in a short period of time.

Use of cTnT hs kit can determine tenfold lower circulating level of Troponin can be detected and hence diagnosis of a micro-infarction.

The values of Troponin T "suspected of AMI" indicated that such results were mostly found among the male patients who were smokers, diabetic, hypertensive or were suffering from CHD (Chronic heart disease).

This study was therefore very much helpful in making decision on the basis of measured levels of Troponin T hs, cardiac enzymes CK and CK-MB in patients coming to emergency department of the hospital for early diagnosis, assessment and management of myocardial damage to patients, with acute myocardial infarction.

Recommendation of this Study: It is recommended that a strict follow up by the patient as well as by the physician, is essential for the patients "suspected of AMI", to minimize mortality and morbidity among local population.

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