EVALUATION OF CLINICAL UTILITY OF SERUM ENZYMES AND TROPONIN -T IN THE EARLY STAGES OF ACUTE MYOCARDIAL INFARCTION.

Hitesh Shah and N. Haridas*

*Department of Biochemistry, Pramukh Swami Medical College, Shri Krishna Hospital and Medical Research Centre, Karamsad, Anand, Gujarat, *Professor of Biochemistry, Smt. N.H.L. Municipal Medical College, Ellisbridge. Ahmedabad.*

ABSTRACT

Laboratory infarction diagnostics are based on the detection of elevated serum activities of total Creatine Kinase (CK), Creatine Kinase isoenzyme MB, (CKMB), Lactate dehydrogenase (LDH), isoenzyme forms of LDH and transaminases. Determination of these cardiac marker enzymes permits a highly sensitive diagnosis of transmural myocardial infarction. In such patients the diagnosis of acute myocardial infarction can be confirmed by the clinical symptoms, and changes in the ECG in addition to the enzyme assays. The 50 AMI patients selected in the present study were those admitted to the ICCU of Shri Krishna Hospital, Karamsad. The blood samples were taken at the time of admission (ie. within four hours of the start of chest pain). The samples were analyzed for CK, CKMB, SGOT, (Serum glutamate oxaloacetate transaminase) α HBDH α - hydroxybutyrate dehydrogenase and troponin T. The serum CKMB activity in AMI showed an increase only 5-6 hours after the commencement of chest pain. The elevation in SGOT and α HBDH was still delayed. At the same time we could observe that the cardiac Troponin T (cTnT) was elevated at the time of admission of the patient itself. This increase of cTnT in AMI patients was 20 times higher than the normal blood donors. The controls included 25 normal blood donors and 25 patients with polytraumatic injuries with no chest contusion. The study shows that cTnT estimation could serve in the early diagnosis of AMI. The increase of cardiac troponin T in AMI patient was 20 times higher than the normal blood donors in AMI patients at the time of admission. Cardiac troponin T in serum appears to be a more sensitive indicator of myocardial cell injury than CKMB activity and its detection in the circulation may be a useful prognostic indicator in patients with unstable angina as well. When the blood of normal blood donors or that of patients with polytraumatic injury was analysed the troponin T values were well within the normal range in both the above categories showing that cardiac troponin T is highly specific for heart tissue. Although CKMB and cardiac troponin T are released soon after the myocardial injury, the release of cardiac troponin T is much earlier than CKMB thereby invalidating the important role of cardiac troponin T in diagnosing AMI. Cardiac troponin T has been shown to be highly sensitive for cardiac injury and not elevated in any other trauma, heavy exercise or skeletal muscle injury. Cardiac troponin T is ordinarily undetectable in healthy individuals, and so its measurement can serve as a powerful tool in the diagnosis of AMI.

KEY WORDS

Cardiac Troponin T, alpha hydroxybutyrate dehydrogenase, Lactate dehydrogenase, myocardial infarction

Author for Correspondence :

Hitesh Shah

*Department of Biochemistry ,
PramukhSwami Medical College,
Shri Krishna Hospital and Medical Research Centre,
Karamsad, Anand, Gujarat, INDIA.,*

INTRODUCTION

The transmural infarction diagnosis can be achieved by ECG changes and the elevation of cardiac enzyme markers. Nevertheless, there are problems with the diagnosis of small myocardial infarctions which are comparable in importance with the transmural infarctions. The ECG offers little help in such cases and the sensitivity of the laboratory parameters like CK, Creatine phosphokinase isoenzyme MB (CKMB), LDH, and Alpha Hydroxybutyrate Dehydrogenase (HBDH) is not adequate because of the variation in normal serum levels and of the short-lasting and small increases in their serum activities after such events. Of course, the same applies to patients with unstable angina pectoris also.

The available diagnostic parameters are also unsatisfactory in patients with multiorgan diseases or with additional skeletal muscle lesions, because in such cases a definite differentiation of skeletal muscle and heart muscle necrosis is impossible because of the organ distribution of CK, LDH or their isoforms. The CKMB values are seen to be elevated only 5-6 hours after the chest pain has begun. Hence an early cardiac marker of AMI shall be greatly welcome.

In addition to the diagnosis of acute myocardial infarction the laboratory parameters are of major importance in monitoring the course of the infarction and in estimating the infarct size. Cytosol proteins such as CK are washed out in different percentages in dependence on the blood flow in the early phase of the infarction and therefore appear earlier in the reperfused infarct, and their plasma activities are higher than in the case of non-reperfused infarcts.

A new immunoassay for cTnT which eliminates most of the earlier shortcomings of laboratory infarction diagnostics was planned to be carried out to evaluate the utility value of Troponin T in early diagnosis of AMI. Such studies have been scanty on Indians.

MATERIALS AND METHODS

The study carried out incorporates the serial estimations of different cardiac enzyme markers in the sera of patients having myocardial infarction. They were admitted to the ICCU of Shri Krishna Hospital, Karamsad. The first blood sample was collected at zero hours (i.e. at the time of admission of the patient). All the patients admitted within 5

hours of the starting of chest pain were included in the study. 1.5 million units of Streptokinase was mixed with 100 to 150 ml of normal saline and was administered by intravenous infusion over a period of one hour to these patients soon after their admission. The blood samples were collected in vials containing no anticoagulant or preservative. The samples were collected using a disposable needle and syringe and care was taken to get non hemolysed samples. The serum samples were analyzed for CK, CKMB, SGOT, α HBDH and Troponin T. The study comprised of 50 patients with AMI out of which 12 were females and 38 were males. Twenty five subjects were selected from normal blood donors and another 25 subjects with polytraumatic injury without any chest or cardiac ailment were also included in the study to serve as controls. Well standardised methods were used for the estimation of CK, CKMB, SGOT, α HBDH and Troponin T (1-5). The serum Cardiac Troponin T concentration was measured using the reagent kit manufactured by Boehringer Mannheim based on enzyme linked immunoassay.

RESULTS

The results of the present study are given in tables 1 to 3 and graphs 1 to 2.

DISCUSSION

The diagnosis of acute myocardial infarction (AMI) (6) has traditionally been based on the characteristic clinical history, electrocardiographic abnormalities and increased serum concentrations of cardiac marker enzymes. As the differential diagnostic value of chest pain is limited (6) and the electrocardiographic changes have various degrees of sensitivity and specificity (7-9), the measurements of serum enzymes as a reflection of damage to myocardial muscle cells still play an important role in the diagnosis of AMI. Measurements of creatine kinase (CK), aspartate aminotransferase, and lactate dehydrogenase are well-established methods for this. Considerable effort has been made in recent years to improve the specificity and sensitivity of methods for diagnosing AMI. Myoglobin is an early and sensitive marker of cardiac cell damage but lacks specificity (10). The use of LDH isoenzyme 1 improves specificity in diagnosing AMI; however, this isoenzyme is not restricted to cardiac muscle tissue, and increase in its serum concentration has been observed in noncardiac conditions also (11). Clinicians would

therefore benefit from the introduction of a new cardiac specific marker of damage to myocardial muscle cells. The contractile and regulatory proteins of the myocardium provide such a useful diagnostic tool. Troponin T (TnT) is a part of the troponin complex in striated muscles, where it binds the troponin complex to tropomyosin. Although both cardiac and skeletal muscles contain TnT, the amino acid sequence of the protein in the two types of muscles differs (12,13), making it possible to raise antisera against cardiac-specific TnT. The high specificity and sensitivity of cardiac TnT in diagnosing and monitoring AMI had been demonstrated by Johannes Mair *et al* (14).

Several epidemiological studies have established various factors which increase the risk of developing myocardial infarction. The cardinal risk factors identified in AMI are hypercholesterolemia, systemic arterial hypertension, smoking, obesity, sedentary habits and consumption of a largely high saturated fat diet. In the present study smoking was found to be associated with 18.4% of patients with acute myocardial infarction. Amongst the male patients seven were chronic smokers. All of them were below 50 years of age. This might point towards the deleterious effect of smoking in causing early AMI. Our study is in agreement with Paul M. Ridker (1) who found 13.9% of patients in his study were smokers.

Troponin T is a part of the regulatory system of the contractile complex of skeletal and heart muscle. It is expressed in two different isoforms, namely skeletal muscle troponin T and cardiac troponin T. After loss of the integrity of the cell membrane, it is released into the circulation similar to myoglobin or creatine kinase (8). Because cardiac troponin T is ordinarily undetectable in healthy individuals (9) its measurement is a powerful tool in the diagnosis of acute myocardial infarction. Moreover, about one-third of patients with unstable angina pectoris show increased cardiac troponin T in serum (10). These patients may be a subgroup with a high risk for complications i.e. acute myocardial infarction (11). After myocardial cell necrosis an increased concentration of cTnT is observable in blood for more than a week (12). Thus cardiac Troponin T measurement is particularly useful in clinical circumstances in which traditional enzyme determinations fail to diagnose myocardial cell damage efficiently (13-15). Given the high sensitivity of cardiac Troponin T measurements, the

authors (16) were interested to know whether determination of cTnT may be useful for the noninvasive detection of myocardial cell damage in patients with acute myocarditis. The diagnosis of myocardial muscle cell damage is generally based on the patient's clinical history, electrocardiogram and enzyme concentrations in serum, especially creatine kinase isoenzyme MB. The concentration of CKMB is higher in myocardium than in other tissues, but the protein is also present in skeletal muscles (17). Pathological values for CKMB in serum can therefore be seen in some instances of skeletal muscle damage e.g. with heavy physical exercise (18). Skeletal muscle damage has been postulated to be the most likely reason for increased CKMB in serum of athletes, where the possibility of simultaneous release of CKMB from both heart and skeletal muscle could not be excluded.

Among many available serological tools for detecting acute myocardial infarction, the determination of the MB isoenzyme of creatine kinase provide the highest diagnostic efficiency (16-19). Therefore, CKMB determinations are generally regarded as the reference standard for diagnostic tests for AMI. Yet variable normal serum levels of CKMB (20-22) and its brief elevation in serum during the course of myocardial infarction (23-25), limit the diagnostic value of CKMB determinations. In practice, physicians are sometimes left with patients in whom definitive diagnosis of myocardial infarction cannot be made by CKMB measurements and an alternative test of even greater sensitivity and specificity is needed. The data of their study indicate that the newly developed troponin T test improves the efficiency of serodiagnostic tools for the detection of myocardial cell necrosis as compared with conventionally used cardiac enzymes (25). Our study is in good agreement with the above observations and further confirms the utility value of cardiac troponin T in AMI detection.

In our study of 50 patients with AMI, 42 showed only slightly elevated levels of CK ($p < 0.005$) at the time of admission (at zero hours), but eight patients had CK values within the normal range. At the same time in all the patients, the CKMB values were within the normal range at the time of admission ($p < 0.05$) showing that for very early detection of AMI (i.e. within 3 to 4 hours after the onset of chest pain), the CKMB alone may not be helpful. When CK and CKMB estimations were carried out at 6 hours after the admission of the patient both were found to be

significantly increased ($P < 0.001$). CKMB elevation in our study took place only six hours after the admission of the patient. There was further increase of CKMB at 14 hours.

Further in our study the pattern of changes in the concentrations of AST and α HBDH showed variation from that of CK and CKMB. Both AST and α HBDH were also normal at zero hours. (i.e. at the time of admission). The AST and α HBDH values started rising at six hours and exhibited further rise at 14, 32 and 48 hours. These observations of ours are comparable to the studies reported by other authors (26).

According to Katus *et al* (1993) Troponin T is elevated more than twice the analytical sensitivity of the assay (0.5 ng/ml) 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. The diagnostic efficiency of troponin T was superior to that of CKMB and remained elevated for 5½ days after admission. In our study the troponin T values were found to be increased in all the patients at zero hours at the time of admission of the patient. Whereas CKMB levels were within the normal limits. We also observed that there was a steep rise in

the troponin T values at 6 and 14 hours after giving streptokinase to the patients showing very good reperfusion. Further, we made the following observations from our studies.

- The increase of cardiac troponin T was 20 times higher than the normal blood donors in AMI patients at the time of admission.
- Cardiac troponin T in serum appears to be a more sensitive indicator of myocardial cell injury than CKMB activity and its detection in the circulation may be a useful prognostic indicator in patients with unstable angina as well.
- When the blood of normal blood donors or that of patients with polytraumatic injury was analysed the troponin T values were well within the normal range in both the above categories showing that cardiac troponin T is highly specific for heart tissue.
- Although CKMB and cardiac troponin T are released soon after the myocardial injury, the release of cardiac troponin T is much earlier than CKMB thereby invalidating the important role of cardiac troponin T in diagnosing AMI.

Table 1

CK, CKMB, SGOT, α HBDH and Troponin T estimations in normal blood donors and polytraumatic injury

		CK (U/L)	CKM B (U/L)	SGOT (U/L)	α HBDH (U/L)	Troponin – T (ng/ml)
Control	Blood Donors	85.4 $\pm$ 30.5 N = 25	8.5 $\pm$ 5.1 N = 25	19.1 $\pm$ 9.4 N = 25	39.2 $\pm$ 26.5 N = 13	0.09 $\pm$ 0.18 N = 25
	Patients with polytraumatic injury	102.6 $\pm$ 37.7 N = 25	14.9 $\pm$ 8.6 N = 25	25.6 $\pm$ 9.1 N = 25	139.5 $\pm$ 43.7 N = 25	0.09 $\pm$ 0.04 N = 25

Table 2

CK, CKMB, SGOT, α HBDH and Troponin T estimations in AMI patients at the time of admission

CK (U/L)	CKMB (U/L)	SGOT (U/L)	α HBDH (U/L)	Troponin-T (ng/ml)
218.4 $\pm$ 222.9 N = 49	20.1 $\pm$ 18.8 N = 49	42.4 $\pm$ 40.3 N = 49	192.4 $\pm$ 141.7 N = 38	2.04 $\pm$ 1.96 N = 49

N = No. of subjects
Age = 43 to 75 years

Table 3

CK, CKMB, SGOT, α HBDH and Troponin T estimations in AMI patients at six hours

CK (U/L)	CKMB (U/L)	SGOT (U/L)	αHBDH (U/L)	Troponin-T (ng/ml)
826.5 $\pm$ 722.0	98.8 $\pm$ 72.7	60.6 $\pm$ 63.4	282.3 $\pm$ 232.2	4.97 $\pm$ 4.74
N = 43	N = 43	N = 43	N = 34	N = 43

N = No. of subjects
Age = 43 to 75 years

Graph - 1: The comparison of normal blood donors with AMI patients at the time of admission and at 6 hours after the admission.

Graph - 2: The comparison of normal blood donors with patients at the time of admission and at 6 hours after the admission.

REFERENCES

1. Neumeier, D. (1996) Activity kinetics and Diagnostic Significance in Myocardial Infarction. *Clinica Chimica Acta* 73, 445-451.
2. Hafkenschied, J. C. M. (1979) *J. Clin. Chem. Clin. Biochem.* 17, 219.
3. Remppis, A., Scheffold, T., Karrer, O., Zehelein, J., Hamm, C., Grunig, E., Kubler W., Katus, H.A., (1994) Assessment of reperfusion of the infarct zone after acute myocardial infarction by serial cardiac troponin T measurement in serum, *Br. Heart J.* 71(3), 242-248.
4. Lee, T.H.H., Rouan, G.W. and Weiberg, M.C. (1987) Sensitivity of routine clinical criteria for diagnosing myocardial infarction within 24 hours of hospitalization. *Ann. Intern. Med.* 106,181-6.
5. McQueen, M.J., Holder, D. and El Maraghi, N.R.H. (1983) Assessment of accuracy of serial electrocardiograms in the diagnosis of myocardial infarction. *Am. Heart J.* 105, 258-61.
6. Behar, S., Schor, S., Kavir, I., Barrel, V. and Modan, B. (1977) Evaluation of electrocardiogram in emergency room as a decision making tool. *Chest* 717, 486-91.
7. Rude, R.E., Poole, W.K. and Muller, J.E. (1983) Electrocardiographic and clinical criteria for recognition of acute myocardial infarction based on analysis of 3697 patients. *Am. J. Cardiol.* 52, 936-42.
8. Drexel, H., Dworzak, E., Kirchmair, W., Miltz, M.M., Puschendorf, B. and Diestl, F. (1983) Myoglobinemia in the early phase of acute myocardial infarction. *Am. Heart J.* 105, 642-51.
9. Chan, K.M., Ladenson, J.H., Pierce, G.F. and Jaffe, A.S. (1986) Washington University Case Conference. Increased creatine kinase MB in the absence of acute myocardial infarction. *Clin. Chem.* 32, 2044-51.
10. Eisenberg, E. and Kielley, W.W. (1974) Troponin-tropomyosin complex. *J. Biol. Chem.* 249, 4742-8.
11. Katz, A.M. *Physiology of the heart.* New York: Raven Press (1977), 99-101.
12. Johannes Mair, Erika Arther-Dworzak, Peter Lechleitner, *et al.* (1991) Cardiac Troponin T in Diagnosis of Acute Myocardial Infarction. *Clin. Chem.* 37, 845-52.
13. Basil, L. and William, G. (1988) Usefulness of a rapid initial increase in plasma creatine kinase activity as a marker of reperfusion during thrombolytic therapy for AML. *Am. J. Cardiol.* 62,20-24
14. Adams, J.E. III, Abendschein, D. R. and Jaffe, A.S. (1993) Biochemical markers of myocardial injury. Is MB creatine kinase the choice for the 1990's? *Circulation* 88, 750-3.
15. Gerhardt, W., Katus, H. A., Ravkilde, J. and Hamm, C. W. (1992) S-Troponin-T as a marker of ischemic myocardial injury (Letter). *Clin. Chem.* 38, 1194-5.
16. Katus, H. A., Remppis, A., Neumann, F.J., Scheffold, T., Diederich, K.W. and Vinar, G. (1991) Diagnostic efficiency of troponin T measurements in acute myocardial infarction. *Circulation* 83, 902-12.
17. Hamm, C.W., Ravkilde, J., Gerhardt, W., Jorgensen, P., Peheim, E. and Ljungdahl, L. (1992). The prognostic value of serum troponin T in unstable angina. *N. Engl. J. Med.* 327,146-50.
18. Hossein-Nia, M., Mascaro, J., McKenna, W. J., Murday, A.J. and Holt, D.W. (1993) Troponin T as a non-invasive marker of cardiac allograft rejection (Letter). *Lancet* 341, 838.
19. Donnelly, R. and Hillis, W.S. (1993) Cardiac troponin T (Letter). *Lancet* 341, 410-1.
20. Irvin, R.G., Cobb, F.R. and Roe, C.R. (1980) Acute myocardial infarction and MB creatine phosphokinase: Relationship between onset of symptoms of infarction and appearance and disappearance of enzyme. *Arch. Intern. Med.* 140, 329-334.
21. Ljungdahl, L., Gerhardt, W. and Hofvendahl, S. (1980) Serum creatine kinases B subunit activity in diagnosis of acute myocardial infarction. *Br. Heart J.* 43, 514-522.

22. Elser, R.C. and McKenna, K. (1981) Creatine kinase B-subunit activity in human sera: Temporal aspects of its sensitivity after myocardial infarction. *Clin. Chem.* 27, 57-60.
23. Koch, T.R., Mehta, U.J. and Nipper, H.C. (1986) Clinical and analytical evaluation of kits for measurement of creatine kinase isoenzyme MB. *Clin. Chem.* 32, 186-191.
24. Shell, W. E., DeWood, M. A., Kligerman, M., Ganz, W. and Swan, H.J.C. (1981) Early appearance of MB-creatine kinase activity in nontransmural myocardial infarction detected by a sensitive assay for the isoenzyme. *Am. J. Med.* 71, 254-262.
25. Hugo, A. and Katus, M.D. (1991) Diagnostic efficiency of troponin T measurement in Acute Myocardial Infarction. *Circulation* 83, 902-912.
26. Baardam, T., Hermens, W. T. and Lenderink, T. (1996) Differential effects of tissue plasminogen activator and streptokinase on infarct size and rate of enzyme release. *Eur. Heart J.* 17(2), 237-45.