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Rapid Diagnosis of Acute Myocardial Infarction

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ABSTRACT

To improve the specificity of biochemical markers of myocardial infarction (MI) a method to measure cardiac troponin-I (CTn-I) was developed. CTn-I is a protein unique to cardiac muscle and is released after MI. Consecutive 150 patients admitted to the coronary care unit was studied. Value of CTn-I was determined in all samples. CTn-I concentration in the MI patients group was increased compared to that in the control [18.7 +/- 0.13 (mean +/- SD, n = 100) vs. 5.31 +/- 0.13, n = 50] «g/l. Measurement of CTn-I accurately detects MI in patients and should facilitate the diagnosis and management of such patients. *Iran. Biomed. J. 3 (1 & 2): 59-62, 1999*

Keywords: Cardiac troponin-I, myocardial infarction, creatine kinase

INTRODUCTION

CTn-I is a 23.5 kDa. protein unique to cardiac muscle and is released after MI [1]. Several studies have showed that CTn-I is more effective test for MI than creatine kinase isoenzyme (CK-MB) and lactate dehydrogenase (LDH) [1-3]. Since CTn-I is not found in skeletal muscle, it is highly specific for myocardial injury and is not elevated after muscle injury or in renal failure [3]. With the availability of rapid automated immunoassays, serum CTn-I is likely to replace CK-MB, and other enzymes as the marker of choice for the diagnosis of MI [1-3]. Clinical studies have shown that assay of cardiac troponin may challenge the role of CK-MB in diagnosis of MI [2, 3]. Although measurement of CTn-I is more specific for detection of cardiac injury than the measurement of CK-MB, its sensitivity and specificity relative to CK-MB for detection of MI has not been established [3]. Detection of cardiac injury in patients with chest trauma is difficult because the level of the CK-MB can be elevated from skeletal muscle injury alone [4]. CTn-I is structural protein of the thin filament. High concentration of this protein in blood is indicative of MI and minor myocardial injury [5]. The incidence of myocardial injury defined by elevated levels of CTn-I was unexpected high and associated with increased morbidity and mortality [6]. Cardiac isotype of the myofibrillar contractile protein, troponin I, is located specifically in the mammalian heart [7]. Troponin T is the tropomyosine-binding protein of the troponin regulatory complex located on the filament of the contractile apparatus [8]. CTn-I is more cardiac specific than the CK-MB and remains increased longer than levels CK-MB [9]. Thus, while CK-MB is weathering the storm, the jockeying for position continues for the next generation of markers of MI [10]. The purpose of this study was to determine a very sensitive biochemical indicator of MI patients.

MATERIALS AND METHODS

Study population. Consecutive patients (n=100) admitted to the Shahid Beheshti Hospital were prospectively enrolled. For each patient, either the history, or the clinical situation suggested the possibility of myocardial ischemia. Blood samples were obtained at least every 12 h for measurement of CTn-I. Subjects were divided into the following two groups:

Group 1: Control subjects. Fifty healthy volunteers (30 men and 20 women) between 25-65 years, with no history of cardiovascular disease, were used as control group. These subjects were deemed free of clinical complication or medications on the basis of interviews.

Group 2: Patients with MI. Hundred patients (60 men, 40 women) between 30 to 65 years. The diagnosis, based on criteria established by the World

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Health Organization, included typical or atypical chest pain, unequivocal changes in the electrocardiogram. Single MI was subsequently confirmed from ECG criteria, that is, appearance of pathologic Q waves accompanied by an elevation of the ST segment and subsequently inversion of the T wave together with significant elevations in serum glutamic oxalooacetic transaminase, CK-MB and LDH levels. Blood samples were drawn from groups 1 and 2.

Samples were drawn into tubes with no preservatives, and centrifuged at 3500 ×g for 20 min, and stored at -20°C. Serum samples and standards (100 µl) in duplicate were incubated with (600 µl) conjugate solution (20 mmol of citrate and 50 mmol of phosphate pH 6.5) in the streptavidin - coated tubes for 45 min at 20°C. The tube contents were aspirated and the tubes were rinsed two times with tap water. The (600 µl) substrate solution (50 mmol/l phosphate citrate buffer, pH 5.2, containing sodium perborate 4.1 mmol/l and 20 µg/l standard of CTn-I) was added to the tubes and incubated for 45 min at 20°C. Absorption was read at 405 nm. CTn-I values were calculated from the calibration curve. A new calibration curve was constructed for each assay. A standard curve was constructed using various concentration (0-30 µg/l) of a stock standard solution of CTn-I.

Statistical analysis. Sensitivity and specificity were calculated for CTn-I relative to the clinical diagnosis. Receiver operating characteristic (ROC) curve was developed for CTn-I. Students's *t*-test was used for between group comparison of results, *P<0.05* were considered to indicate statistical significance.

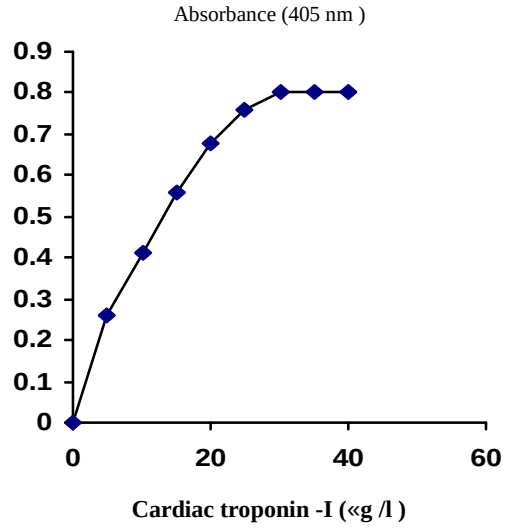

Fig. 1. Standard curve for cardiac troponin ,each point represents the mean value +/- SD of 10-12 experiments, each assay in duplicate (*P< 0.05).*

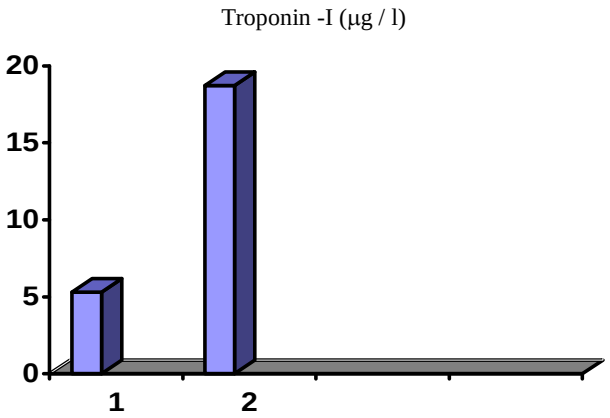

Fig. 2. Cardiac troponin -I levels in normal human serum (1) and MI patient serums (2). Each columns represents the mean value +/- SD of 10 -12 experiments (*P <0.05).*

RESULTS

The measuring range of CTn-I concentration extended from 0 -30 µg/l. Figure 1 shows a typical standard curve for the assay of cardiac CTn-I. The CTn-I concentration in the normal subjects and the patients reported in Figure 2. In patient group cardiac troponin-I levels were elevated above the normal range. Figure 3 shows the times for CTn-I appearance. In all of the patients studied, only one peak was observed. Distribution of CTn-I values in the normal subjects and the patients showed in Figure 4. To establish the discriminator limit of CTn-I for MI in this study ROC curves were constructed the number true positive (sensitivity) and false positive (specificity) obtained with serum CTn-I at different values (Figure 5).

Analytical sensitivity linearity and recovery: A human serum sample with high amount of troponin was diluted step-wise with normal serum. The data show high linearity of CTn-I measurements in the assay. To assess analytical recovery, various amounts of human CTn-I was added to the human normal serum. The values show good agreement with the theoretical value. The lowest detection limit for CTn-I was 0.005 µg/l. Results show that the CTn-I assay is linear up to at least 20 µg/l. The recovery values for a blank serum sample supplemented with high CTn-I was 99 % expected CTn-I concentration of 15 µg/l.

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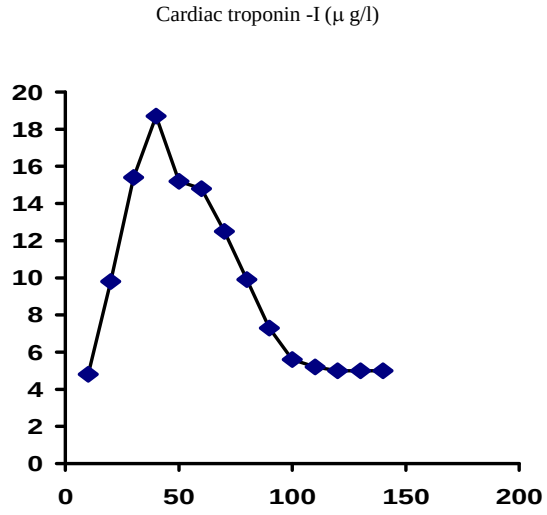

Fig. 3. Cardiac troponin -I release profile after myocardial infarction. Each point represents the mean value +/- SD of duplicate samples (P <0.05).

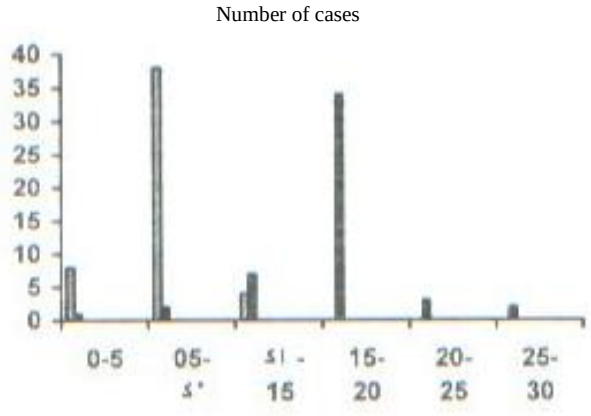

Fig. 4. Distribution of troponin-I values in normal subjects (1, dark), and MI patient (2, light). Each column represents the mean value +/- SD of 10-12 experiments. Each assayed in duplicate (*P<0.05*).

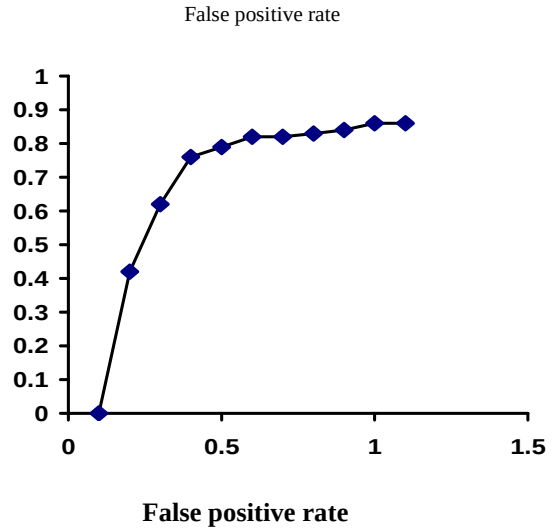

Fig. 5. ROC curve for values of cardiac troponin-I. Each point represents the mean value +/- SD (*P<0.05).*

DISCUSSION

Results obtained from this paper, like those of other authors [1-3] suggest that CTn-I assay can be used in MI patients. Also, confirm other reports [4, 5] that CTn-I remains abnormally increased in serum for a much longer time after MI than levels CK-MB. The average maximal value of CTn-I all patients was at least 3 times greater than the normal subjects, significantly greater than the increase of CK-MB, which partly explains the high diagnostic sensitivity of present assay for CTn-I . The profiles of CTn-I values after MI were in general similar for the all patients studied. The time courses were roughly monophasic in all of the patients studied. CTn-I measurements by this newly developed assay appear to be more sensitive than measurements of serum cardiac enzyme activities, and it seems that minor MI which was undetectable by electrocardiogram recordings may be detectable by CTn-I measurements. Elevated levels of this long lived marker is highly specific for cardiac injury, equally as sensitive as elevated levels of CK-MB and highly corrected with development of new areas of regional dysfunction determined by echocardiography. Despite its structural location, CTn-I levels were elevated within 5 to 20 hours after infarction, not significantly different from the earliest detectable rise of sarcoplasmic CK-MB isoenzyme. These findings suggest that CTn-I has several advantages over traditional serological markers of myocardial injury. The greatest advantage is its cardiospecificity: CTn-I measure-ment is, therefore, particularly helpful in the assessment of patients with myocardial ischemia and skeletal muscle damage. These results like other authors [1, 4, 10] suggest that CTn-I assay can be used in patients with unstable angina to discern subtle pathophysiological aspects of myocardial damage. The unique properties of this marker and, in particular, its high diagnostic specificity (86%) and sensitivity (95 %), the high concentration gradient between MI and normal blood. The assay of non-invasive and myocardial specific CTn-I, which indicates early

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cardiac involvement and dysfunction will, therefore, be helpful in management. Finally, the determination of CTn-I in combination with early markers of MI, such as LDH and CK-MB allows a reliable diagnosis of MI. The choice of CTn-I as an innovative biochemical marker for the diagnosis of MI has led to the development of a new rapid one-step assay that is very sensitive and highly specific.

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