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ORIGINAL ARTICLE

Comparison of Mass Versus Activity of Creatine Kinase MB and Its Utility in the Early Diagnosis of Re-infarction

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Abstract Currently employed markers for the detection of acute coronary syndrome are Troponin T, CK (Creatine Kinase) and CKMB activity. CKMB activity measured by immunoinhibition method can give falsely elevated results due to the presence of atypical CK and CKBB and at times lead to the mis-diagnosis of acute coronary syndrome. Hence, CKMB mass (CKMB) measured by electrochemiluminence sandwich principle was employed. In this cross-sectional study 183 samples of 61 patients were analyzed within 6 h of diagnosis of acute coronary syndrome and followed up to 72 h. The correlation coefficient between CKMB activity and CKMBM at 4–6 h was 0.744, while at 12–24 h it was 0.909 and at 48–72 h it was 0.337. Thus there was good association between the two methods at 12–24 h but, statistically for method comparison studies and for replacing one method by another, the two methods need to be in agreement with one another. In this study the two methods are not in agreement with one another and thus analytically not replaceable. Another finding was obtained that CKMBM reached cut off levels prior to CKMB enzyme activity and hence, CKMBM is clinically better than CKMB activity to detect reinfarction.

Keywords Acute coronary syndrome · CKMB enzyme activity · CKMB mass

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Introduction

Acute coronary syndrome is a constellation of clinical symptoms caused by myocardial ischemia. It encompasses a spectrum of coronary artery diseases, which include unstable angina, ST-elevation myocardial infarction (STEMI) and non-STEMI (NSTEMI).About 32 million deaths in the world are attributable to non-communicable diseases and more than half of these are because of Cardiovascular Disease (CVD) and more than one-third of these deaths occur in the middle-aged adults [1]. About half of the world's cardiovascular yoke is predicted to occur in the Asia–Pacific region [2]. Deaths from coronary heart disease in India rose from 1.17 million in 1990 to 1.59 million in 2000 to 2.03 million in 2010 and expected to be 2.58 million by 2020. In India 52 % of cardiovascular deaths occur below the age of 70, compared with 23 % in countries with established market economies [3, 4]. Cardiac troponin is the preferred marker of myocardial infarction but in case of its non availability CKMB mass (CKMBM) is employed for the diagnosis as per the universal definition of myocardial infarction by American Heart Association [5]. The shortcoming of cardiac troponin is its long half-live, hence CKMBM is the preferred marker for the detection of re-infarction after an index event when cardiac troponin levels are still raised [6]. The activity of CKMB in the serum can be influenced by light, temperature, pH and prolonged storage [7, 8]. CKMB assay is performed by immunoinhibition method which measures the catalytic activity of the CKMB isoenzyme, in this method anti CK-M antibody is used which inhibits M subunit of CKMB and CKMM, eventually enzyme activity of CK-B is measured. In this technique the B-subunit of CKBB and atypical CK are also measured as these are not inhibited and could give falsely high results [9, 10]. Thus a new assay was devised known as CKMB mass, in which CKMB was

measured in terms of its protein concentration rather than its biological activity and CKMB mass has been measured by electro chemiluminescence technology, sandwich principle where monoclonal antibodies against CKMB have been used so specifically the CKMB fraction is measured. Inspite of the fact that more sensitive methods have been established for the diagnosis of myocardial infarction, measurement of CKMB by immunoinhibition method is still being performed in many laboratories and is a matter of concern since misleading diagnosis of myocardial infarction can be made due to the presence of atypical forms of CK such as macro CK and CKBB [11]. Hence, this study was undertaken to perform a clinical and analytical comparison of the CKMB enzyme activity (CKMBE) and the CKMBM concentration in patients with acute coronary syndrome.

Materials and Methods

This study consisted of 183 samples of 61 patients above the age of 25 years who were admitted to the emergency department and the cardiac intensive care unit of Shree Krishna hospital within 6 h of signs and symptoms of acute coronary syndrome. Patients with renal diseases, acute stroke and myopathy were excluded from the study CKMBE activity > 25 % of total CK activity is suggestive of myocardial damage and while CKMBM more than 10 ng/mL is indicative of myocardial infarction [12].

Study Design

A prospective study was designed to select serum samples from these patients on admission (within 6 h), after 12–24 h and after 48–72 h of signs and symptoms of acute coronary syndrome because the release of markers depends on the time of onset of necrosis we looked at the indices in relation to the time since the onset of necrosis and the damaged myocardial tissue releases CKMB in a characteristic fashion i.e. it rises in 4–6 h, peaks in 12–24 h and returns to normal in 2–3 days [13].

Blood was collected from all the enrolled patients within 1 h of admission and Troponin T and CKMBM were measured quantitatively using a ECLIA (electro chemiluminescence immune assay) based on electro chemiluminescence technology, sandwich principle. (Cobas e411, Roche, Mannheim, Germany) while, CK and CKMBE was analyzed on Cobas Integra 400 plus using Roche Method according to the recommendations of the International Federation of Clinical Chemistry (IFCC), the Société Française de Biologie Clinique (SFBC), the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (SCE), the Société Française de Biologie Clinique (SFBC), and the Deutsche Gesellschaft für Klinische Chemie (DGKC) by immunoinhibition method.

Statistical Analysis Plan

Association between CKMB activity and CKMBM was made at all the time frames and their correlation co-efficient was determined, agreement between the two methods was found at all the three time intervals by using Bland–Altman graphs, line diagram showing the trend of enzyme kinetics was done and clinical superiority of CKMBM over CKMB activity to diagnose reinfarction was found out. Statistical analysis was done using SPSS software version 14 and Microsoft excel.

Results and Discussion

There were 61 patients in this study, out of which 51 were males and 10 females. The association between CKMBE and CKMBM was fair at 4–6 h, with correlation co-efficient of 0.744 (Fig. 1a). At 12–24 h the association between CKMBE and CKMBM was good, with correlation co-efficient of 0.909 (Fig. 1b) The association between CKMBE and CKMBM was poor at 48–72 h when the values were supposed to reach cut off levels with correlation co-efficient of 0.337 (Fig. 1c).

Agreement between CKMB activity and CKMBM was established by Bland–Altman plot. When two methods are compared they need to show good agreement so as to be comparable and replaceable by one another. It is quite unlikely that different methods will agree exactly hence we need to know how much the new method differs from an existing one and how it affects clinical interpretation. As the procedure removes most of the variation between the methods and leaves the measurement of error it is expected that the differences will be normally distributed.

At 4–6 h the limits of agreement were 64.9, −64.9 with a mean difference of 0 between CKMB activity and CKMBM (Fig. 2a). At 12–24 h the limits of agreement were 94.4, −94.4 with a mean difference of 0 (Fig. 2b) and at 48–72 h the limits of agreement were 56.9, −56.9 with a mean difference of 0 (Fig. 2c). At lower values the difference is in negative bias, and bias becomes positive when the activity value increases. Since the limits of agreement at all three time intervals are so wide hence, the two methods are not analytically replaceable.

The trend of CKMB activity and CKMBM has been shown. It is evident that at 48–72 h, CKMBM reaches cut of levels while CKMB activity does not. Hence, clinically CKMBM is better than CKMB activity in detecting re-infarction (Fig. 3).

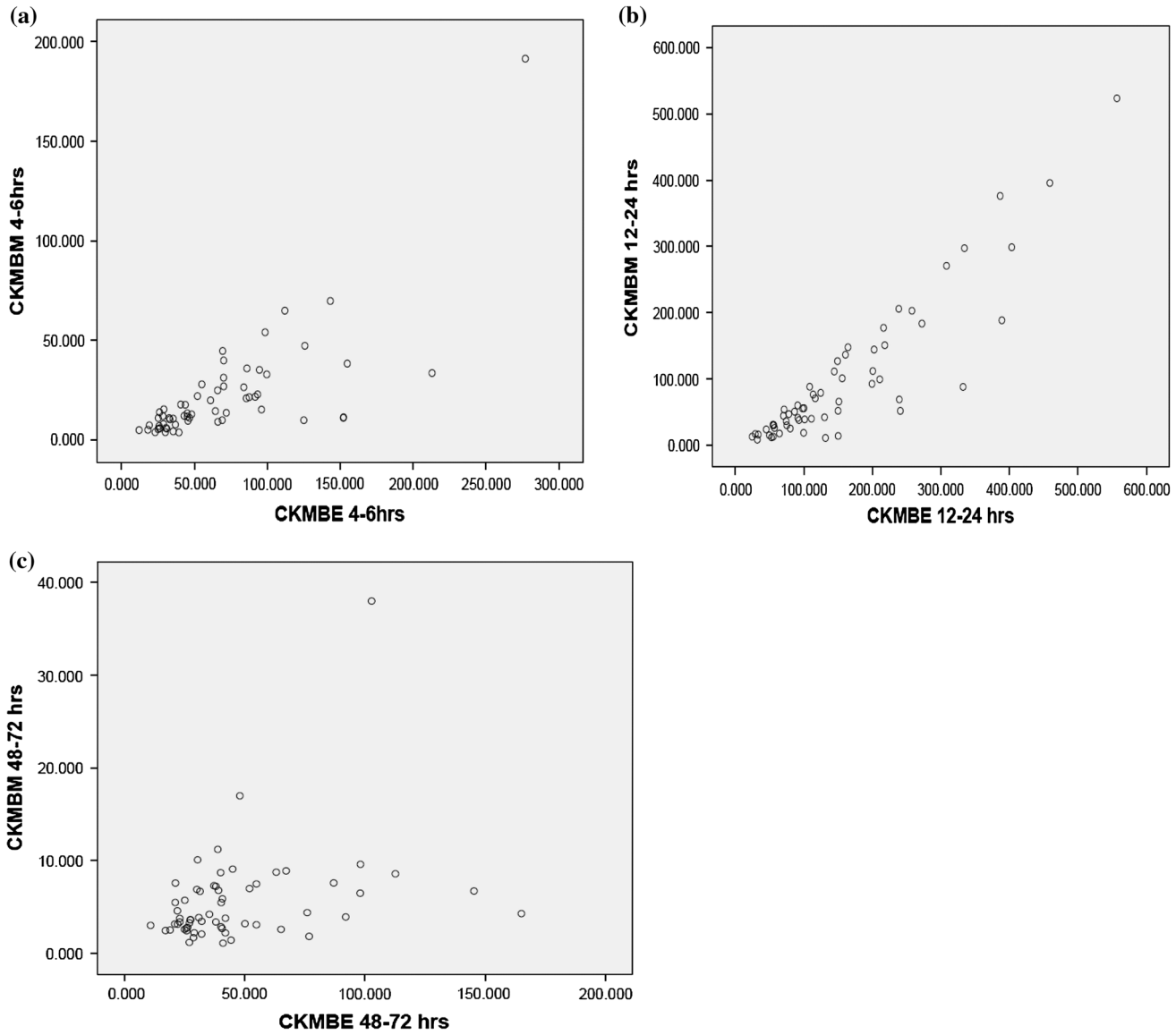

Fig. 1 **a** Association between CKMBE and CKMBM at 4–6 h. **b** Association between CKMBE and CKMBM at 12–24 h. **c** Association between CKMBE and CKMBM at 48–72 h

The chances of detecting re-infarction by measuring CKMB mass after 48–72 h is more as compared to CKMB activity because in 80.7 % patients CKMBM reached the cut off levels while CKMB activity did not. Of the total number of 61 patients, in 46 patients CKMBM reached lower cut off levels at 72 h while CKMB activity did not (range of CKMB activity varied from 27.5 U/L to 145.28 U/L which is higher than the cut off levels) and in 11 patients both CKMBmass and activity reached lower cut off levels. Therefore, clinically CKMBM is better than CKMB activity in detecting re-infarction (Table 1).

Discussion

In this study, initially the correlation between the two method was fair ($r = 0.744$), while at 48–72 h there was poor correlation ($r = 0.337$) between the two methods. At the peak levels there was good correlation ($r = 0.909$), which was similar to studies performed by Eisenberg et al. [14] and Seo et al. [15].

Eisenberg et al. compared CKMB activity and CKMBM in 1,298 samples from 226 patients with acute myocardial infarction CKMB activity was performed by immunoadsorption

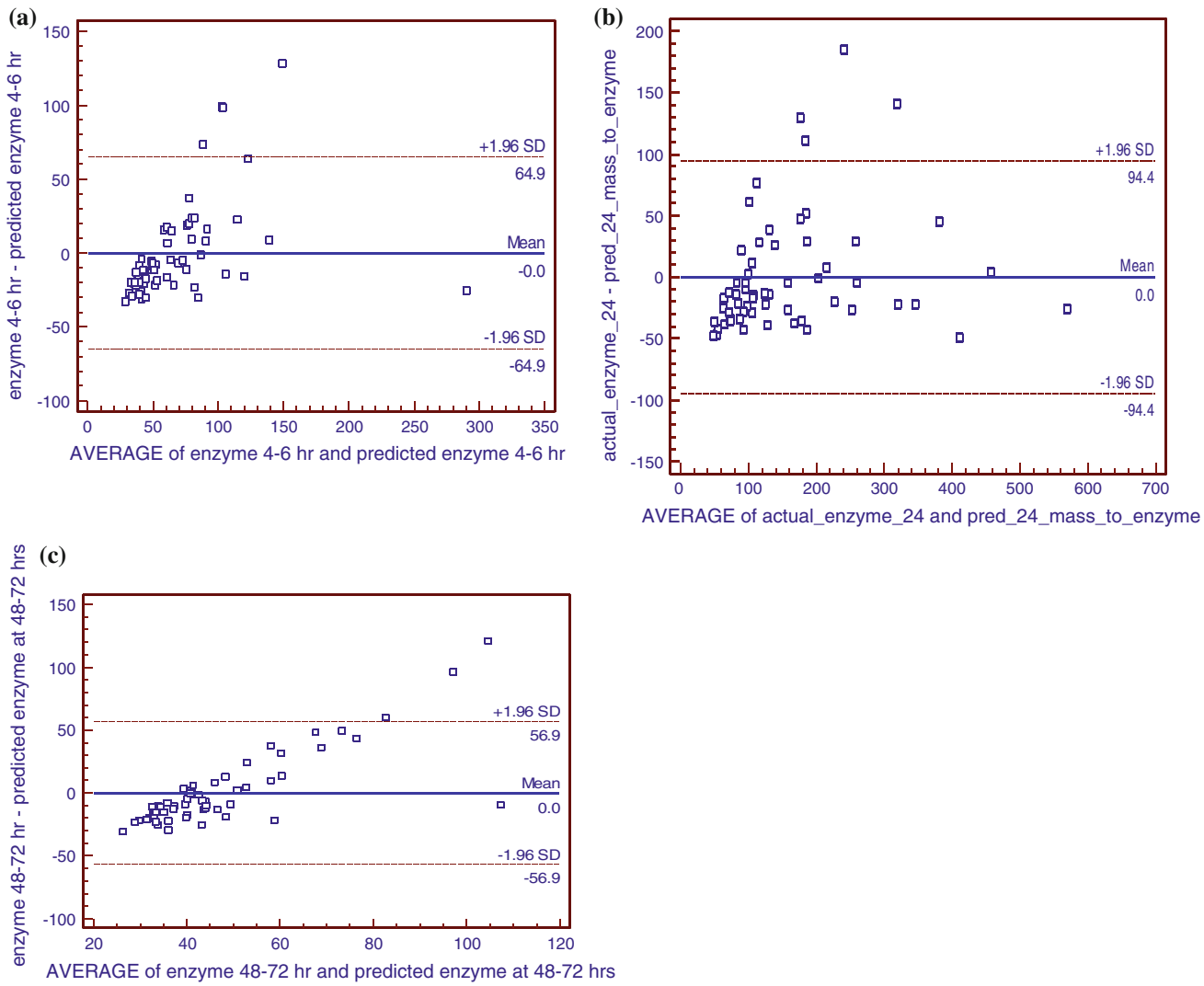

Fig. 2 **a** Agreement between CKMBE and CKMBM at 4–6 h. **b** Agreement between CKMBE and CKMBM at 12–24 h. **c** Agreement between CKMBE and CKMBM at 48–72 h

Table 1 Total no. of cases that touched cut off levels of CKMBE and CKMBM at 48–72 h

	CKMBM		Total
	<10 ng/mL	>10 ng/mL	
CKMBE			
<25U/L			
Cases	11	0	11
Percent	19.3	0	18
>25U/L			
Cases	46	4	50
Percent	80.7	100	82
Total			
Cases	57	4	61
Percent	100	100	100

CKMBM CKMB mass, *CKMBE* CKMB enzyme activity

assay and CKMBM by immunochemiluminometric assay and reported that these two assay correlated well with correlation coefficient of 0.94 and thus are comparable and the concordance was 96 %. But they measured the first sample

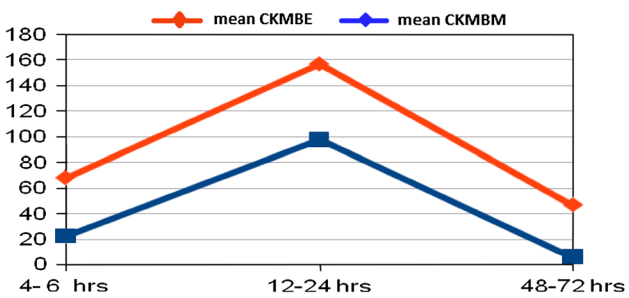

Fig. 3 *Line* diagram showing trend of CKMBE and CKMBM

on admission (which ranged from 0–12 h) after onset of infarction while in our study we have obtained the first sample within 6 h of onset of symptoms and were analyzed up to 72 h which gives us a better picture of how they reach cut off levels.

Seo et al. carried out a study using 312 samples of 20 patients with acute myocardial infarction and compared CKMB activity and CKMBM in them. CKMB activity was done by immunoinhibition and CKMBM by immunochemiluminometric assay. A good correlation was obtained between the two methods with correlation coefficient of 0.94.

Though these studies have stated that for two methods to be comparable they should have good correlation. But correlation coefficient is a measure of association that is the strength of relation between the two variables and not the agreement between the two. Hence, there will be a perfect correlation if the points lie along any straight line. But there will be perfect agreement if and only if the points are along the line of equality. Hence, when two methods are compared they need to show good agreement rather than good correlation to be comparable and replaceable. It is quite unlikely that different methods will agree exactly hence we need to know how much the new method differs from an existing one and how it affects clinical interpretation [16, 17].

In this study though there is good correlation between the two methods at peak level but there is no agreement between the two methods neither at 4–6 h, at 12–24 h nor at 48–72 h. Hence, analytically the two methods are not comparable.

Also CKMBE is measured by immunoinhibition method in which anti-M antibodies are added which inhibit CK-M subunit and then CK-B is measured, multiplied by 2 and CKMB is obtained. But anti-M does not inhibit CKBB, macro CK type I and mitochondrial CK. These are also measured while performing immunoinhibition method. Hence, this method can give false positive results. While CKMB mass is measured by electrochemiluminescence sandwich principle in which antibodies are added against CKMB and measurement is done.

A number of studies have been done in the past which have stated that the presence of macro CK type I, mitochondrial CK and CKBB in patient's serum can give abnormally high values of CKMB by immunoinhibition assay and lead to the misdiagnosis of acute myocardial infarction [18–20]. As compared to total CK and CKMBE, CKMBM has more sensitivity and specificity to diagnose myocardial infarction [21–23]. It has been proved by Hoshino et al. [24] that sarcomeric and ubiquitous mitochondrial CK isoforms make up about 80 % of measured CKMBEin normal subjects which is not inhibited by immunoinhibition method and incorrectly measured as CKMB activity. Thus the specificity of immunoinhibition method that detects CKMB acitivity is low. Lee et al. [25] has shown the prevalence of macro CK type I to be 0.43 % and mitochondrial CK (macro CK type2) to be 1.2 % these are the cause of low prognostic value of CKMBE, as also he has stated that macro CK isoenzymes may be a source of laboratory interferences. Fleming et al. [26] stated r^2 = 0.004 that is there was no correlation between the two methods. Thus the reason for the false positive values of CKMBE was the presence of CKBB, macro CK type I (MM–MB inter), mitochondrial CK, an iso-form running between CKMB and CKBB(MB-BB inter) which were shown by electrophoresis and drug related effects on CKMB activity analysis.

In our study, in 46 patients i.e. in 80.7 % of the patients CKMB mass reached the cut off levels at 72 h while CKMB activity did not and in 19 patients both CKMB activity and CKMBM reached cut off levels thus CKMB mass reached cut off level prior to CKMB activity which is similar to the findings of Seo et al. [15]. He stated that CKMB protein mass disappeared more rapidly from the serum in immunochemiluminometric assays than did CKMB enzyme activity by immunoinhibition assays. Therefore CKMB mass has higher chances of detecting reinfarction as compared to CKMB enzyme activity. Hence, clinically CKMB mass is better than CKMB activity. The reason for this is that CKMB mass measures both active and inactive enzyme and the degradation rate of inactivated CKMB precedes that of active CKMB, hence the disappearance rate of the mixture of active and inactive CKMB may be more than that of active CKMB alone as also false increase in CKMB activity in immunoinhibition assays may affect the disappearance rate more slowly than in electrochemiluminescent assays.

Conclusion

Thus in this study CKMBE and CKMBM were compared. The correlation between the methods initially was fair, while it was good at the peak levels i.e. at 12–24 h but at 48–72 h it was poor. There was no agreement between the two methods at all three time frames i.e. at 4–6 h, 12–24 h and at 48–72 h. Hence, the two methods cannot be compared and replaced analytically. Comparison was done by Bland–Altman analysis which statistically is the best way for performing method comparison. There have hardly been any studies that have thrown light on the agreement between these two methods, which statistically is the correct way of performing method comparison. In this study an interesting finding was obtained, that CKMBM reached normal limits prior to CKMBE. This implies that a patient having re-infarction can be diagnosed by CKMBM rather

than by CKMB activity and therefore clinically CKMBM is better that CKMBE.

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