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Original Research Paper

Diagnostic Efficacy of Cardiac Isoenzyme CK-MB in Pericardial Fluid for Post-mortem Diagnosis of Myocardial Infarction

[1]Pankaj Suresh Ghormade, [2]Narendra Baluram Kumar, [3]Chaitanya Vidyadhar Tingne,
[4]Ajay Narmadaprasad Keoliya

Abstract

Sudden cardiac death due to acute myocardial infarction is the most prevalent cause of death in adults and constitutes a significant portion of the autopsies that are conducted by Forensic pathologists. Serum cardiac isoenzymes Creatine phosphokinase-MB (CK-MB) has high sensitivity and specificity for cardiac damage and routinely used for clinical diagnosis of myocardial infarction (MI). However, in Forensic Medicine, diagnostic utility of this cardiac marker for post-mortem diagnosis of MI has not been fully established. Present study is carried out with aim to evaluate the diagnostic efficacy of CK-MB in pericardial fluid for autopsy diagnosis of MI. This study included 119 medico-legal autopsy cases selected during a period of two years. Four study groups were formed depending upon final cause of death Biochemical analysis of pericardial fluid was carried out. Highest levels of CK-MB were noted in deaths due to IHD. By this cardiac marker, early infarcts can be predicted in sudden cardiac deaths associated with severe coronary artery disease & inconclusive histopathological findings.

We obtained high diagnostic sensitivity and negative predictive value of CPK-MB for autopsy diagnosis of MI in pericardial fluid comparable to the clinical analyses on serum.

Key Words: Autopsy, IHD, Myocardial infarction, Pericardial fluid, CPK-MB

Introduction:

Ischemic heart disease (IHD) due to coronary atherosclerosis is the most prevalent cause of sudden death in adults over the age of 30 years, but it is not infrequent in younger subjects. [1]

Hence, Coronary artery disease is sometimes called 'The Captain of the Men of death' and it constitutes a significant portion of the autopsies that are conducted by Forensic Pathologists in our country. [2]

'Sudden cardiac death' is defined as, natural death due to cardiac causes, heralded by abrupt loss of consciousness within 1 h of the onset of acute symptoms. [3] In more than 50% of these cases, death is caused due to cardiac arrhythmias induced by myocardial ischemia.

Corresponding Author:

[1]Assistant Professor
Department of Forensic Medicine & Toxicology,
Indira Gandhi Government Medical College,
Nagpur, Maharashtra, India (440018)
E-mail: pankajforensic@gmail.com
[2, 3] Assist. Prof,
[4]Prof &HOD
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When autopsy is performed, there may be presence of coronary atherosclerosis without evidence of demonstrable gross or microscopic findings of myocardial infarction (MI).

As survival period of more than 6 hours is required for changes of MI to appear in the heart, infarction is not apparent on gross examination until 12–24 hrs and light microscopic (H&E) changes are not apparent before 4–6 hrs. [4]

Due to limitations of histopathological findings [5], it is necessary to establish diagnostic utility of different biochemical cardiac markers in biological fluids for postmortem diagnosis of MI. Measurements of CK-MB and other cardiac markers in serum are commonly used for clinical diagnosis of acute myocardial infarction. [6-8]

Various authors have investigated role of CK-MB for post-mortem diagnosis of MI in serum and pericardial fluid (PF). [9-18]Few of them have attempted to determine if post-mortem levels of this isoenzyme is significantly higher in deaths because of myocardial ischemia than those because of other causes of death. [9-13]

However, these studies do not mention the overall diagnostic efficacy of this marker as

mentioned in clinical practice. Hence, in the present study, we had investigated diagnostic efficacy & distribution of CK-MB in PF fluids for autopsy diagnosis of MI as compared to other causes of death.

Material & Methods:

We studied 119 cases (91 males and 28 females) selected from total medico legal autopsies from Dec-2010 to June-2012 conducted at dept. of Forensic Medicine, IGGMC, Nagpur. Mean age of subject was 50.77 years (S.D.10.81, range 30-75 years).

Mean post-mortem interval was 13.50hours (S.D.6.90 hours, range 2-24 hours).All dead bodies were kept in cold compartments prior to autopsy. We have excluded from the study all cases with pericardial disease or haemorrhage in pericardial fluid & cases showing signs of decomposition.

Subjects were assigned into one of the four diagnostic groups depending upon their final cause of death as follows:

- **Group I:** Sudden cardiac deaths due to Ischemic Heart Disease (n=52) subdivided in deaths due to myocardial infarction (n=28) and coronary artery disease (CAD) (n=24). These subgroups were classified upon histopathological confirmation.
- **Group II:** Violent Asphyxial Deaths (n=24) subdivided into deaths due to hanging (n=16) and drowning (n=8).
- **Group III**: Deaths due to Poly-trauma (n=20) included vehicular accident cases with extensive muscle damage without any evidence of chest trauma.
- **Group IV**: Natural deaths not due to cardiac diseases (n=23) included deaths due to pulmonary consolidation (n=10), lung abscess (n=04), non-traumatic intra-cerebral hemorrhage (ICH) (n=04), non-traumatic subarachnoid hemorrhage (SAH) (n=02), sepsis due to infected wounds (n=02) and acute bronchial asthma (01).

Details in each case were obtained from inquest paper, treatment record, death certificate and other relevant documents issued from hospitals in hospitalized cases.

In addition, history particularly important towards diagnosis of cardiac disease was elicited from near relatives or friends. For studying factors affecting CK-MB levels in pericardial fluid, we have included cases of poly-trauma to analyse effect of muscle damage and cases of violent asphyxia to observe the effect of hypoxia on heart and consequent release of cardiac marker. Whereas, other naturalcauses of deaths in group IV were assigned as controls.

Survival time (ST) was known in 78 cases & mean ST was 1.21 hours (S.D. 1.01 hours, range–0.10-5 hours). All cases in group II were brought dead to the emergency department. Out of total 119 cases, Cardio pulmonary resuscitation (CPR) was attempted in 45 cases. In group I, cardiac complaints were present in 43 cases, 21 patients were hospitalized & ECG findings of IHD's were present in 8 of these cases.

Pericardial fluid samples were collected from pericardial sac by using sterile syringe after incising parietal pericardium. It was then centrifuged immediately at 5000 rpm for 15 minutes & supernatant was collected for enzyme analysis. Standard laboratory procedures were used for measuring levels of CK-MB in pericardial fluid & biochemical analysis was carried out on an automated analyser using commercial kits.

We used Immunoinhibition/Mod.IFCC [8] method of estimation for CK-MB involving principle of UV kinetic reaction. As the PF showed high enzymatic activities compared to clinical range on initial tests, dilution of fluid was carried with normal saline (0.9%) in the ratio of 1:9 before analysis and results then obtained were multiplied by 10.

Detailed gross examination and dissection of the heart was carried out by short–axis and inflow outflow techniques. Only those cases showing severe coronary artery disease i.e. vessel showing ≥ 75% stenosis of lumen on transverse sectioning [19], were included in final observations.

Histopathological examination (HPE) of heart was carried out in each case with haematoxylin and eosin (H&E) staining. On HPE, findings of MI were divided into acute, healing and healed infarct.

Hearts showing presence of coagulative necrosis with various degrees of nuclear changes and prominent infiltration of neutrophils were diagnosed as acute MI (AMI). Whereas, cases in which heart showed, above changes in various stages with presence of mononuclear leucocytes and fibroblasts without neutrophils were considered as healing MI (HMI).

When, HPE of hearts showed presence of collagenous scarring without cellular infiltration, cases were labeled as old healed MI (OHMI). Presence of features like inter-fibrillar edema/interstitial edema, patchy eosinophilia were considered as inconclusive and these cases were assigned into deaths due CAD.

For statistical analyses of the data, the MedCalc version 13.1.0.0 program was used. Probability level $p<0.05$ was considered significant. Non-parametric tests i.e. Kruskal–Wallis test & Mann–Withney test (rank–sum test) were used to compare levels of CK-MB in PF amongst four diagnostic groups & to compare pair of diagnostic groups, respectively.

In addition, specific contrasts for variable grouped by diagnostic category were carried out using Mann–Withney test. Receiver operating characteristic (ROC) curve [20, 21] was used for measuring area under the curve for CK-MB ,to obtain its cut-off level for evaluating diagnostic efficacy and to discriminate between cases died due of IHD's and non -IHD by using diagnostic cut off value.

Observations & Results:

All 52 cases included in group I, had severe coronary artery atherosclerosis in one or more major epicardial arteries and triple vessel disease pattern was predominantly(48.07%) seen. Coronary thrombosis was found in seven cases of acute MI. (Table 1)

The values (Mean±Standard error of mean, S.D. and range) obtained for CK–MB in each diagnostic group. (Table 2) Highest levels were observed in death due to IHD's as compared to cases in other diagnostic groups. Non-parametric Kruskal–Wallis test (Table 2) was used for comparing differences in CK-MB level in PF amongst all diagnostic groups. We have observed statistically high significant difference in activities of CPK-MB ($P=0.0001$) amongst all four diagnostic groups.

In group I, statistically non-significant ($P=0.6729$) differences were observed in values (Table 3) of CK-MB between cases of definite MI (n=28) & cases with CAD (n=24). On the Mann-Withney test (Table 4), we observed highly significant ($P<0.0001$) levels of CK-MB in cases of death due IHD's as compared to deaths due to violent asphyxia, poly-trauma and other natural deaths excluding cardiac causes.

Receiver-operating Characteristic (ROC) Curve Analysis:

For discriminant analyses, we used cause of death as grouping variable, total 119 cases in all groups were divided into deaths due to IHD's (n= 52) and that of due to non IHD's (n=67).ROC curve (Fig.1) was established by taking levels of CK-MB as an independent variable with paying special attention to the area (Table 5), which represents the correct diagnosis in two individuals, one with MI and one without MI.

By using ROC curve, we determined the diagnostic cut-off value of 979 U/L for CK-MB in pericardial fluid (Table 5) for post-mortem diagnosis of myocardial infarction in cases of IHD's. At this diagnostic cut off level, we have obtained sensitivity=94.23% NPV=94.1% & specificity=71.64% of CK-MB for diagnosis of MI. No statistically significant correlations were observed between the levels of CK-MB and postmortem interval period included in the study and the use of cardiopulmonary resuscitation.

Discussion:

As mentioned in literature, following acute myocardial infarction, the initial CK-MB rise occurs 4 to 6 hours after the onset of chest pain, peaks at 24 hours, and returns to baseline at 48 to 72 hours (t1/2 of CK-2 is 10 to 12 hours). One advantage of CK-MB over other markers is that it remains elevated for longer periods and it is easier to detect re-infarction using serial measurement. [6-8]

In clinical practice, measurement of CK-MB level in serum is routinely used to detect myocardial ischemia. However, use of this cardiac marker in serum for post-mortem diagnosis of MI has limitations due other factors affecting enzyme levels. [11, 13] Hence, we chose pericardial fluid over other biological fluids because, it is an ultra-filtrate of plasma [22, 23] hence, biochemical analysis can be done by using kits standardized for serum.

It lacks RBC's, therefore does not show hemolysis phenomenon that frequently interfere with biochemical determinations in serum. And as pericardial and myocardial irrigations are shared, markers of myocardial ischemia are detectable in PF before they are detectable other biological fluids. [9-18]

We obtained highest values of CK-MB in group of deaths due to IHD's as compared to other diagnostic groups.

Statistically non-significant differences were noted in levels of CK-MB between cases of AMI and cases with inconclusive H&E findings classified as deaths due to severe coronary atherosclerosis in group I. This signifies utility of performing CK-MB test on pericardial fluid for post-mortem diagnosis of early MI, because histopathological finding may be inconclusive in such cases. This finding from our study concurs with the findings of earlier studies. [13, 25]

On Kruskal–Wallis test, highly significant differences were observed for CK-MB amongst all diagnostic groups. On Mann-Withney test, we observed statistically significant levels of CK-MB in group of subjects who died of IHD's (Group-I) in comparison to other groups

represented by the subjects who died due to violent asphyxia (Group-II), poly-trauma (Group-III) and natural deaths excluding cardiac causes (Group-IV).

Findings of our study are in accordance with ones that reported in earlier studies. [9, 10, 15, 17, 25] However, Barabas B [18] found non-significant difference in CK-MB levels between asphyxial deaths and death due to AMI citing intense agony prior to death as probable reason for conflicting results.

On ROC curve analysis, we have observed significant areas under the curve for CK-MB (0.848). Diagnostic cut-off point value obtained for CK-MB showed very high sensitivity and negative predictive value and only 3 cases out of 52 included in IHD's, had values less than cut-off obtained.

These are the cases of MI in healing phase, suggestive of probable decline in enzyme levels after its initial peak during acute phase. Whereas, 19 cases of non-IHD out of 67 were incorrectly classified leading to lower specificity for the marker.

This might be due to other factors affecting its levels, as out of those 19 cases, nine corresponded to violent asphyxia deaths in which there might well have been an intense agony with consequent acute myocardial suffering involving the release of different markers into the cadaver, another seven had died from poly-traumatism in which, too, cardiac traumatism may have been involved. T

hree deaths were from natural causes (2-ICH and 1-SAH) that showed presence of severe coronary artery disease and death may occur due to cardiac arrhythmias in these disease conditions. [26, 27]

We obtained higher diagnostic sensitivity and NPV for CK-MB on ROC curve analysis as compared with the results of Carceles-Perez et al. [10]In clinical practice, sensitivity of the serum CK-MB for diagnosis of MI is from 92 to 100 at 3 hours from the onset of symptoms, whereas diagnostic specificity has been reported to be very close to 100%. [28]

Conclusion:

As we obtained nearly equal diagnostic sensitivity and negative predictive value for the cardiac marker CK-MB in pericardial fluid compared to clinical analysis on serum sample, hence, this cardiac marker in pericardial fluid could be of great help for autopsy diagnosis of MI in combination with histopathological examination.

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Fig.1: ROC Curve of CPK-MB levels in Pericardial Fluid

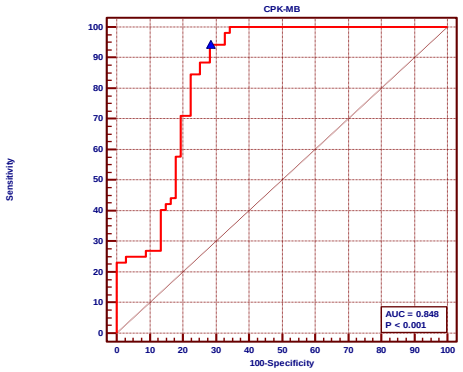

Table 3: Difference in Levels of Enzymes in Definite MI & CAD Cases in Group-I (Mann–Withney Test)

Group I (IHD's)	CPK-MB (U/L)	
Myocardial infarction (n=28)	Mean	5055
	SD	5968.75
	Median	2113.1
	Range	497.82 – 18943.6
Coronary artery disease (n=24)	Mean	4144.77
	SD	4050.5
	Median	2405.5
	Range	1018 – 15618
	Z statistics	0.422
	P value	0.6729

Table 4: Mann–Withney test Between Diagnostic Groups

Variable	Comparison groups	Z statistic value	P value
CPK-MB	I – II	4.101	0.000
	I – II	4.564	0.000
	I – IV	5.538	0.000

(Groups: I–IHD, II-Violent asphyxia, III-Poly-trauma, IV-Natural deaths other than cardiac disease)

Table 1
Histopathological Findings of Heart in Diagnostic Groups

Groups	Total cases	AMI or HMI*	AMI+ OHMI**	OHMI	MF#	Interstitial edema	Congestion
I (IHD)	52	AMI-19 HMI-4	5	14	2	46	52
II (Violent asphyxial deaths)	24	-	-	2	0	10	24
III (Poly-traumatic deaths	20	-	-	-	0	0	20
IV(Non-cardiac natural deaths)	23	-	-		3(2ICH, 1SAH)	3	23

*HMI-healing MI, **OHMI-old healed MI, #MF-myocardial fibrosis

Table 2
Pericardial Fluid Levels of CPK-MB and Results of Kruskal-Wallis Test in the Diagnostic Groups

Parameter	Levels	I. IHD	II. Violent asphyxia	III. Poly-trauma	IV. Non cardiac natural deaths	Kruskal-Wallis test
CPK-MB(U/L)	Mean ±SEM*	4635.36± 713	1623.16± 442.3	1088.2± 280.9	858.67±313.5	d.f#=3 test statistics (t)=48.50 P-value=0.0001
	S.D.	5144.92	2166.99	1256.12	1503.65	
	Median	2176.9	458.75	396.5	310	
	Range	497.82- 18943	78 – 8480	52- 4780	115 -6280	

*SEM: standard error of mean, #: degree of freedom

Table 5
Area under Curve & Diagnostic Cut-Off Levels of CPK-MB Established Using ROC Curve Analysis

Variable	AUC*	P	SE**	Lower limit	Upper limit	Cut-off level	Sensitivity	Specificity	NPV#
CPK-MB	0.848	0.001	0.036	0.771	0.907	979 U/L	94.23%	71.64%	94.1%

*Area under curve, ** Standard error, #Negative predictive value