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Comparative Analysis Of Cardiac Iso Enzyme CK-MB In Pericardial Fluid And Serum In The Post Mortem Diagnosis Of Myocardial Infarction.

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ABSTRACT

The measurement of serum CK-MB isoenzyme is a very sensitive and specific indication of myocardial injury since only myocardium has substantial amounts of CK-MB. Serum CK-MB levels are most helpful clinically when the total creatine kinase is nonspecifically elevated, as with intramuscular injections, cardiac catheterization, stroke, noncardiac surgery and electric cardioversion. Elevations of serum CK-MB occurring in Duchenne's muscular dystrophy and other neuromuscular disorders may be due to the presence of abnormal regenerative skeletal muscle fibers, which are known to contain large amounts of CK-MB isoenzyme. These examples emphasize that under normal, nonregenerative conditions, elevations of serum CK-MB are rare in the absence of myocardial injury. We suggest that elevated cTnI and CK-MB levels in blood and pericardial fluid are related to ischemic, hypoxic and/or cytotoxic myocardial damage, which are characteristic of the cause of death, although the levels increase after death depending on myocardial damage at the time of death.

Keywords: Autopsy, IHD, Forensic pathology, myocardial infarction, pericardial fluid, CKMB.

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INTRODUCTION

Coronary artery disease leading to myocardial infarction and other heart conditions will cause more than 25 million deaths over globe in a year. Among non communicable diseases, coronary artery disease will be the top and it will overtake other communicable diseases which causes death and will become first rank killer [1]. India accounts 16-17% of worlds population and coronary artery disease accounts one fourth of death rate here. As a result of westernization in food and life style we Indians have highest prevalence of CAD among various ethnic group living all over world [2]. The term sudden cardiac death which includes all death by natural cause due to pathology in heart, causing acute symptoms like chest pain, giddiness or abrupt loss of consciousness and resulting in death within one hour from onset of symptoms. Some school of study consider the time lag upto one day. Myocardial infarction due to coronary artery ischemia are often the common cause leading to sudden cardiac death. Death is usually due to cardiac arrhythmias particularly ventricular fibrillation caused by myocytes irritability stimulated by ischemia [3]. During autopsy of such cases, coronary atherosclerosis of varying percent in either one or both coronaries will be seen without any evidence of gross or microscopic changes which are usually seen in coronary artery disease leading to MI [4]. Deaths seen several hours after onset of symptoms due to acute myocardial infarction are mainly due to self neglect or misunderstanding the pain as due to gastritis leading to death in unexplained way [5]. Those death which occurs after several hours can be revealed by microscopic examination of the affected tissue and may be seen in naked eye if the time lag is more than 1day [6]. Usually in autopsy of such sudden cardiac death cases, evidence for myocardial infarction is collected usually based on the atherosclerotic plaque occluding either one or both coronaries main or its branches, and also histopathological examination will give positive finding for myocardial infarction only after a significant time lag between onset of symptoms and death might be around 12hours [7]. Waiveness of myocardial fibres will be seen around 6hours after onset of symptoms and also neutrophils infiltration into the interstitial can only be noted at 12hours after the symptoms starts. Anyhow, signs of irreversible injury in the myocardium can be seen as early as 3hous in electron microscopy after death. Using enzyme histochemistry loss of lactate dehydrogenase can be seen at 5 hours after infarct [8]. In order to evaluate and diagnose the infarction early, several studies have laid much importance in application of cardiac markers to diagnose AMI [9]. Biomarkers in blood with increased sensistivity to detect myocyte necrosis makes to arrive diagnosis of MI in 30% of individuals who might have missed to diagnose in other conventional ways. Once cardiac biomarkers importance realized among clinicians, various markers were used like AST, CPK, CK-MB, LDH, Troponin T. Among these markers CK MB and Troponin gained much importance in diagnosing AMI as their sensitivity and specificity were more and reliable than others [10]. In the following thesis the level of CK-MB is compared with pericardial fluid and ventricular blood in the sudden cardiac death cases for the diagnosis of AMI.creatine kinase also called creatine phosphokinase is an enzyme involved in the metabolism of many body tissues and cells [11]. Creatine kinase is involved in the following reaction where creatine gets converted to phospho creatine (PCR) in which Adenosine triphosphate (ATP) gets consumed and adenosine diphosphate gets released along with phospho creatine (PCR). This entire conversion is reversible and hence ADP and PCR can generate back ATP. Tissues of body which uses more ATP like skeletalmuscle, brain, photoreceptor cells of retina, inner ear hair cell, spermatozoa and to lesser extent smooth muscle, phospho creatine used as base and gets rapidly converted to creatine and releases ATP [12].

MATERIALS AND METHODS

This is prospective study and the whole study was conducted in the Institute of Forensic Medicine, Madras Medical College Chennai-3 for a period of six months in year 2016 .This study quantitatinely analyzed the heart blood from pericardial fluid and in left ventricle for CK-MB level in sudden cardiac deaths within one day after death, by highly sensitive immuno turbidic metry method and correlated with the gross appearance of heart and its dissection.Study sample size is 20 cases of sudden cardiac death. A control group of 10cases consisting of death due to road traffic accidents, poisoning, hanging and cancer patients etc. Ethical clearance obtained from Institutional Ethical committee. In dead bodies with history of

non traumatic sudden death and preferably with history of cardiac symptoms prior to death are selected for autopsy. After making a longitudinal incision on the skin, dissecting out the skin, subcutaneous tissue and muscle with a BP handle scalpel blade, reflecting out the skin to gether with the muscle, cutting the ribs at the costochondral junction along with sternoclavicular joint disarticulating it and the sternum lifted up, dissected out exposing the thoracic cavity. The pericardial sac is then opened with care and the heart lifted for pericardial fluid. Using 21G needle with sterile syringe the pericardial fluid collected. Care should be taken by not puncturing deeper and obtaining different sample. By same technique blood drawn from left ventricle. The collected blood transferred to sterile test tube corked with a stopper. This sample is sent for analysis in a private lab.CK-MB level is estimated by an method called immunoturbidimetry method. Total CK level and CK-MB level obtained. The Heart then removed, washed with running water and weighed . Gross examination of the entire heart was done to look for any presence of scar due to old infarct, hyperemia or any other morbid condition. Serial transverse section involving full thickness of heart was made at a distance of 1cm each from apex to the AV groove. Slices were examined for old fibrotic scar. The heart then dissected along line of flow of blood, observed for raised atheromatous plaque on the inner surface of root of aorta, narrowing of coronary ostia, and narrowing of lumen of coronaries by plaque or thrombus.

Inclusion Criteria

The inclusion criteria comprised an un embalmed adult body with cause of death un explained or with cardiac history or symptoms.

- All cases with history of non traumatic sudden cardiac death subjected for autopsy within 1day of death.
- All dead bodies diagnosed and treated for AMI and died within 1day of time from onset of infarction till autopsy.
- RTA and Natural death cases as a control.

Exclusion Criteria

- Dead bodies subjected to autopsy after 1day of time
- Cases of septicemia, kidney disease, lung disease and CCF are excluded.
- Dead bodies which are showing any signs of decomposition are excluded.
- Cases of any cardiac intervention done are excluded.

There are various factor which alters the out come in this study. post mortem interval which strongly affects the level of CK-MB.

In this study most cases are brought dead to casualty and hence time of death is not known. The post mortem interval given in master chart is only the approximate time since death derived from the information given by the investigating officer.

RESULTS

The study MI sample contained predominantly men 70% and women 30% where as in control group both men and women were 50%. Among the age group, the study cases contained 15% in the group of 20-40yrs, 40% in the group of 41-60yrs and 45%in the group of 61-80yrs. In control cases 40% case was in age group 20-40yrs, 40% in the age group 41-60yrs and 20% in the age group of 61-80yrs. In the control sample 5 cases died due to road traffic accidents, 1case due to poison, 1case due to fall form height, 1case due to hanging, 1case due to liver disease and 1case due to cancer. In all the study cases (n=20) and control cases (n=10) the total CK was estimated in IU/L. Then the CK-MB was estimated separately in left ventricular blood and in pericardial fluid. When the CK-MB level was more than 20% of total CK then the case was considered to be of MI. The normal Total CK was 100-200 IU. Some school of study lower the normal limit of total CK to 70 IU/L.In the study cases, 15 cases out of 20 showed positivity of MIin left ventricular blood, where as 16 cases showed positivity of MI

in pericardial fluid.Sensitivity of the study group in ventricular blood is 75% whereas in pericardial fluid it is 80%.

In the control group, 6 cases out of 10 showed negative for MI in left ventricular blood, whereas 7 cases showed negativity for MI in pericardial fluid. Specificity of the control group in ventricular blood is 60% while it is 70% with pericardial fluid.

Table :1 Sex Distribution Among The Stuy Sample (N=20)

Sex	Frequency	Percentage
Male	14	70%
Female	6	30%
Total	**20**	**100%**

Table 2: Study Group (N=20) Results

S. No	Total CK in IU/L	CKMB in IU/L		Inference
		Left Ventricle	Pericardial Fluid	
1.	220	65	72	Increased in Both
2.	200	58	60	Increased in Both
3.	245	62	63	Increased in Both
4.	410	100	96	Increased in Both
5.	180	45	50	Increased in Both
6.	600	140	164	Increased in Both
7.	170	20	18	Normal
8.	257	58	54	Increased in Both
9.	120	16	20	Normal
10.	300	64	70	Increased in Both
11.	110	12	26	Elevated in Pericardial Fluid
12.	96	10	12	Normal
13.	280	58	66	Increased in Both
14.	584	100	116	Increased in Both
15.	200	42	40	Increased in Both
16.	240	58	62	Increased in Both
17.	89	10	11	Normal
18.	210	50	53	Increased in Both
19.	234	64	64	Increased in Both
20.	372	83	100	Increased in Both

Table 3: Control Group (N=10) Results

S. No	Total CK in IU/L	CKMB in IU/L		Inference
		Left Ventricle	Pericardial Fluid	
1.	350	80	86	Increased in Both
2.	82	10	11	Normal
3.	310	94	80	Increased in Both
4.	264	80	26	Increased in ventricle blood
5.	86	5	8	Normal
6.	406	92	86	Increased in Both
7.	100	10	9	Normal
8.	110	8	12	Normal
9.	79	6	9	Normal
10.	134	12	11	Normal

DISCUSSION

Sudden death is defined as those death which occur within 24 hours of onset of presenting illness. Among sudden death cases, death due to cardiac reason tops the list and among sudden cardiac death, death due to atheroscelerosis accounts for 80% cases [13]. Establishment of cause of death in cases of sudden cardiac death is a difficult task to the forensic pathologist. Difficulties are due to early myocardial infarction death and micro infarcts [14]. Usually it takes 24 hours to see visible gross change in myocardium in case of MI and even microscopic change becomes evident after 6-8 hours of infarction, but most sudden cardiac death occurs within minutes to few hours after onset of infarction. In such cases diagnosis of MI becomes difficult and hence cardiac markers plays a vital role in diagnosis of such cases [15]. Among the cardiac markers used, CKMB and Troponin gained significant advantage in diagnosing MI. Measurement of such marker in blood to detect injury to cell is straight forward and requires few factors.

- Sensitivity – Abudance in cell
- Sepcificity – Wide distribution
- Sample timing – Half Life

The aim of this short study was to measure CKMB level in ventricular blood and in pericardial fluid in cases and control groups and to compare its value and to predict which sample has an higher sensitivity and specificity in diagnosing MI [16].

Limitations of this study include factors like Cohort size, autolysis, variation in time since death and cold storage duration. Inspite of all, the data was analysed that may clear our understanding of cardiac marker CKMB [17].

Even under normal condition it is inevitable that all bodies subjected for postmortem examination will show some degree of autolysis which causes rise in CKMB than its premortem levels. Some other study have reported levels similar to living persons and have ruled out the role of hemolysis and autolysis.[18]

Although this was a small study CKMB level in left ventricle and pericardial fluid were statistically significant [19].

CONCLUSION

This study showed a strong relationship between postmortem CKMB level with death due to myocardial infarction. As like any study, here also there may be few, false positive and negative depending upon cut off value, logistic variation or by any increased time interval. We also should be aware of the normal value of CKMB in various standard lab, control samples elevated level and the nature of the sample taken. In conclusion out of the study group 20 numbers, CK-MB found elevated in ventricular blood for 15 cases and in pericardial fluid for 16 cases. In test group out of 10 cases, 6 cases found negative in ventricular blood and 7 cases in pericardial fluid. Plotting the datas, the sensitivity and specificity was found to be higher in CK-MB values obtained from pericardial fluid than ventricular blood in the diagnosis of MI. Therefore, more large-scale studies are needed before definite conclusion can be drawn from these assays.

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