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Reliability of Post Mortem Pericardial Fluid Troponins for Diagnosis of Myocardial Infarction

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Abstract

Introduction: The purpose of this study was to evaluate the diagnostic role of postmortem pericardial fluid cardiac troponins in cases of sudden cardiac death and to determine its significance.

Methods: This study included totally 30 cases, of which 23 were cases of sudden cardiac death and 7 were control cases. In the following cases, along with age, gender, as well as circumstance of death, level of cardiac troponin I In pericardial fluid, cytological analysis of the fluid as well as post mortem findings of the coronary arteries were all analyzed. Levels of troponin I were assessed with the help of a rapid card test which worked on the principle of sandwich immunoassay. It detects a minimum amount of 0.5ng/ml of cTnI in samples.

Results: In the 30 cases among which the study was conducted, the mean age was 44 years of age, with the majority of the cases lying between the ages of 41–50 (37%). It was also found that the majority of them were males (86.7%) as compared to females (13.3%). The mean time interval between death and sample testing came up to 11 hours, with the majority of the cases being between 16–20 hours time interval (37%). On correlation of rapid card test results and gross post mortem findings, the test showed a sensitivity of 86.7% and specificity of 20%.

Conclusion: From statistical analysis it could be inferred that the frequency of MI was more in males than females, and in the age group of 41–50. The rapid card test had an appreciable sensitivity, but the specificity inferred there could be various situations in which there could be elevated troponins in pericardial fluid. This infers that while a case of myocardial infarction has a higher chance of showing elevated levels of cardiac troponin in pericardial fluid, a case of sudden death can not be reliably diagnosed as AMI on the basis of elevated troponins alone. There must be correlation between clinical, histopathological and cardiac marker findings.

Keywords: Myocardial infarction; Pericardial fluid; Cardiac troponin; Post mortem.

Introduction

Sudden death is one of the most common cases received by any forensic department, in which the dead body is obtained almost immediately after death. The most common cause of this is MI, as its mortality rate is almost equal to or more than 50%. Sudden death is defined as a natural, rapid and unexpected event occurring in an hour or less than an hour from the onset of clinical occurrence in people with unknown or known illnesses in apparently healthy condition.

Myocardial infarction occurs when myocardial ischemia, a diminished blood supply to the heart, exceeds a critical threshold. If ischemia is maintained at this critical threshold level for an extended period, the result is irreversible myocardial cell damage or death. Critical myocardial ischemia can occur as a result of increased demand for the metabolic requirements of the myocardium, insufficient

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oxygen and nutrients supplied to the myocardium via the coronary circulation, or both.[1] Myocardial infarction is known to be most commonly caused by atherosclerosis and subsequent thrombosis of the coronary arteries supplying the myocardial muscle. Significant coronary artery stenosis, defined by invasive coronary angiography, is taken to be >50% decrease in lumen diameter in the left main stem, >70% in other arteries, or between 30–70% with fractional flow reserve <0.80.[2]

The development of an atheroma in the coronary arteries, the rupture of "vulnerable plaques" and the cascade of thrombotic events that follow result in the thrombosis of the artery thus compromising the blood supply to the heart leading to a state of infarction. A vulnerable plaque is defined as a plaque which as compared to other plaques, has higher chances of rupturing and triggering thrombotic events.[3]

Determining cause of death and proving with utmost certainty that the deceased had died of means other than natural causes, are the most important tasks a forensic specialist can perform during the course of an autopsy. There are certainly cases of sudden death which cannot be confirmed to be cases of MI due to lack of definite gross or histological evidence. On top of that, microscopically visible lesions require 6 hours of survival after the coronary event. And for changes to be appreciated macroscopically, minimum 12 hours of survival is required after the acute coronary event. In such cases, where cause of death cannot be determined, myocardial biomarker estimation can be utilised to confirm or exclude MI. There may even be scope for cardiac troponins in the determination of severity of MI as well as prognosis.[4]

Atherosclerosis of the arteries supplying the blood to the heart is what ultimately leads to ischemia and ultimately infarction. accordingly, looking at the degree of the blockage of the coronary arteries on gross examination constitutes a major part in the detection and diagnosis of MI. Studies have established that coronary microvascular disease (CMD)is an early form of atherosclerosis which ultimately builds up to lead to obstructive coronary artery disease.[5]

Cardiac biomarkers

It has been known for some time that troponins are a marker of myocardial necrosis. However, the exact mechanism behind their release is not yet exactly known. Various means of release have been suggested, such as myocyte necrosis, apoptosis, myocyte turnover, increased cell membrane permeability, intracellular release of degradation products or even via formation of blebs of the cell membrane.[6] Selection of a biomarker: 1) it should be abundant in the myocardium and not present in other tissues. 2) it should have a low or undetectable concentration in the blood in the absence of disease.; 3) adequate and timely release when myocardial damage occurs 4) it should remain detectable in the circulation so as to give a convenient diagnostic window, but not so long as to prevent the detection of complications such as early re-infarction, and 5) high analytical sensitivity and specificity and a short turnaround time.[7] (Fig. 1) Cardiac troponin T and troponin I are the most specific and sensitive laboratory markers of myocardial cell injury, and therefore are integral for the diagnosis of myocardial infarction.[8] Studies have shown that along with electrocardiography and CK-MB levels, troponins can be a reliable measure of diagnosis as well as risk stratification in MI patients.

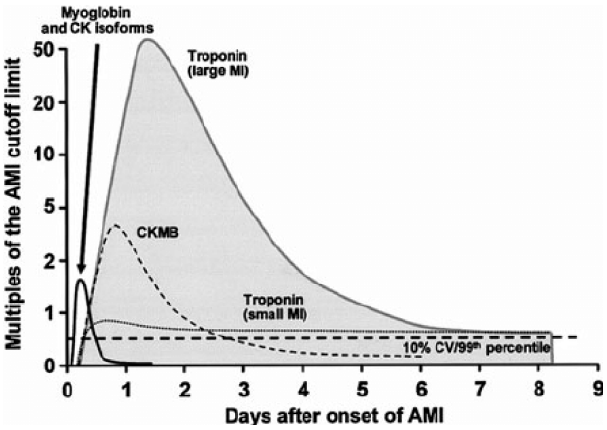


Fig. 1: Cardiac enzyme level variations over time (From "Biomarker of necrosis and cardiac remodelling").

Traditionally, for post mortem myocardial bio marker evaluation (when post mortem interval<48 hours),[9] blood is used. However, significant clotting of the blood sample may occur in the post mortem interval, which may affect values. Using post mortem blood for biomarker analysis also presents its own practical problems, especially during aspiration of the samples. Previous studies have also indicated that cardiac troponin tests were made keeping in mind the utilization of live subject serum for the same, and is unsuitable for postmortem blood.[10]

Estimation of these markers in pericardial fluid, which does not clot over time, eliminates this factor of error.

Objectives of Study

- to analyse the sensitivity and specificity of the rapid card test for cTnI
- to determine the reliability of rapid card test for post mortem diagnosis of MI in cases of sudden death

Materials and Methods

The source of data was 30 cases of sudden onset death of post mortem interval less than 48 hours from the mortuary of Victoria Hospital, attached to Bangalore Medical College and Research institute. A cross sectional study was conducted from the period of July 2017 to March 2019.

Inclusion criteria: cases of sudden death of unknown cause, with post mortem interval being less than or equal to 48 hours.

Exclusion criteria: cases where post mortem interval is more than 48 hours.

After obtaining clearance and approval from the Institutional Ethics Committee, the following study was carried out in Victoria Hospital Bangalore, a tertiary health center which is affiliated to Rajiv Gandhi Institute of Health Sciences (RGUHS). The samples were collected from the dead bodies at the Department of Forensic Medicine mortuary, where the bodies are subjected to autopsy, and the tests are conducted in the Department of Pathology, BMCRI.

Date of obtaining ethical clearance: 06.07.2017

Pericardial fluid samples were obtained by aspiration of the fluid from the pericardial sac of cadavers for which autopsy was done, by the following procedure;

After the ribs were cut and removed to expose the heart in the pericardium as part of the routine autopsy procedure, a small nick was made in the pericardial sac near the apex of the heart with a scissor. The pericardial fluid (about 30–50 ml will be present) was aspirated with a sterile needle and syringe. Around 10ml of pericardial fluid was aspirated.

The samples were subjected to the troponin I rapid card test immediately after extraction from the subject, and results were obtained within 5–10 minutes.

The rapid card test used is of the name "i-tell", a cTnI one step troponin device (mfd by Abo biopharm).

The "rapid card test" method is used to detect the cardiac troponin I in the pericardial fluid samples. This test can be conducted with plasma, serum and whole blood samples.

"Rapid card test" is based on the specificity of the immunochemical reaction between antigens and their corresponding antibodies, for the detection of substances in any fluid sample. Rapid troponin cards are based on the principle of sandwich immunoassay. This test can detect a minimum amount of 0.5ng/ml of cTnI in specimens.

The sample was added to the 'sample pad', which then moves through the conjugate pad. If there are sufficient levels of the cardiac troponins, it combines with conjugate containing the respective antibody (anti-cTnI). The mixture of conjugate and troponin moves towards the test region and reacts with the anti-cTnI antibody coating the test region, thus forming a colored band. A positive card test indicates a sufficiently raised level of cardiac troponin I, which is indicative of Myocardial infarction. A negative card test shows only the control line, (Fig. 2a). a positive test shows 2 lines, the control line and the test line. (Fig. 2b)

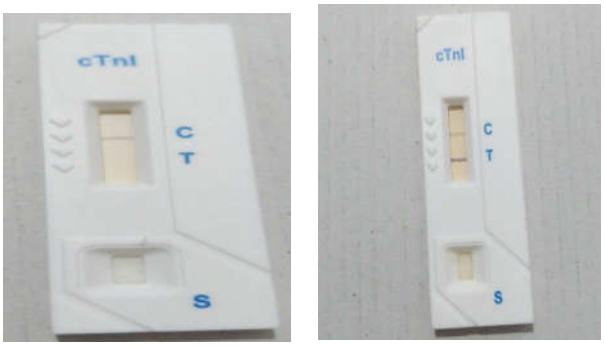


Fig. 2: (a) Negative rapid card test showing only "control" line. (b) Positive rapid card test showing both "control" and "test" lines.

Once the rapid card test was done with the pericardial fluid sample, the postmortem gross findings in the heart were noted. This was in terms of the percentage of blockage in the coronary arteries; and these findings were correlated with the rapid card test (RCT) results and analysed.

Results

Total number of cases: 30

Mean age of the study participants : 44.03 ± 15.49 years (Fig. 3)

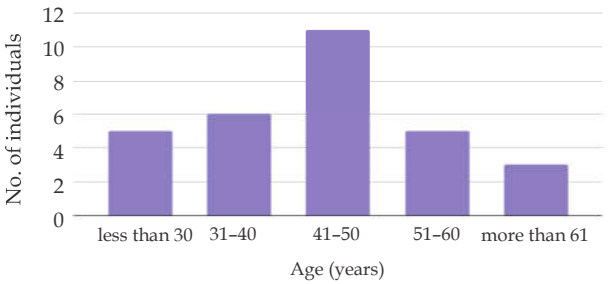
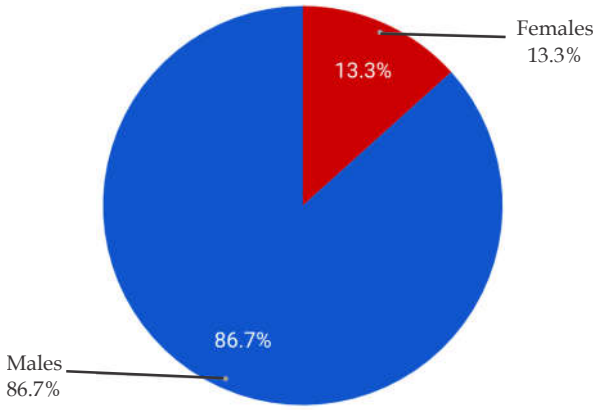


Fig. 3: Mean age of the study participants.

Sex distribution of the study participants

Table 1: Sex distribution of the study participants.

	Frequency	Percent
Female	4	13.33
Male	26	86.67
Total	30	100.0



Graph 1: Sex distribution of the study participants.

Mean time interval in hours = 16.5 hours

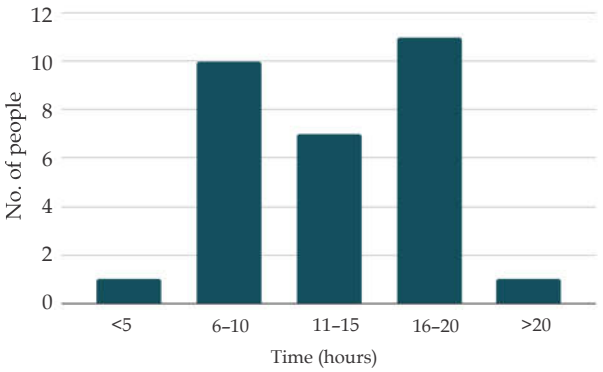


Fig. 4: Time interval in hours among the study participants.

Comparison with rapid card test with significant blockage in coronary arteries.

Out of 30 cases of sudden death, 25 of them showed troponin I elevation.

Out of these 25 cases, 13 cases showed gross evidence of coronary artery blockage. These make up the true positive findings of the study.

The remaining 12 cases which showed troponin I elevation but had no evidence of coronary artery blockage are the false positive findings.

Out of 30 cases, 5 cases showed no elevation of troponin I levels. Out of these 5 cases, 2 showed evidence of coronary artery blockage. These will be considered as the false negatives of the study. The remaining 3 cases which did not show evidence of coronary artery blockage are the true negative findings of the study. (Table 2)

Table 2: Comparison of rapid card test with significant blockage with vessels.

		Significant Blockage		
		Present	Nil	Total
Rapid Card Test	Positive	13 (a)	12 (b)	25
	Negative	2(c)	3(d)	5
Total		15	15	30

(a) would be the true positives (positive RCT in cases with evidence of significant coronary artery blockage).

(b) would be the false positives (positive RCT with no evidence of significant blockage).

(c) would be the false negatives (negative RCT with evidence of significant blockage.

(d) would be the true negatives (negative PCR with no evidence of significant coronary artery blockage).

Pearson's Chi –square – 34.272 p = <0.0

(a) Sensitivity -86.67%

(b) Specificity – 20%

(c) Positive predictive value -52.00%

(d) Negative Predictive Value – 60.00%

(e) Accuracy – 53.33

Discussion

After collecting the pericardial fluid sample and post mortem findings of the patients fitting in the specified criteria over the designated time period, various features were observed. One was the clear gender predisposition, with approximately 87% of the cases being male. (Table 1) The mean duration of the time interval between time of death and time of sample testing came to around 16.5 hours (Fig. 4).

The criterion used in this study fixed the ideal time limit as upto 48 hours from time of death. Studies done by Fox et. al, C. Barberi et. al, Alpert et. al have shown that the levels of cTnI (as well as that of cTnT) are most commonly raised from around 4–9 hours after MI, peaking at 12 to 24 hours.[11–13] According to a study done by C. Barberi et al, it was concluded that after a post mortem interval of 48 hours, the cTnT levels in PCF and peripheral blood were not reliable as they showed slight post mortem elevation after that.[12] According to a study by Zhu BL et al, post mortem time dependant increase of cardiac troponin was most evident in AMI and asphyxiation.[14] The effect of time interval upon the level of troponins in serum emphasise the role it could play in post mortem diagnosis of MI and estimation of time and mechanism of death. It may also have implications in the area of ACS diagnosis and prevention.

After compiling and analysing the results of the rapid card tests for detection of troponin I in the given sample size, a sensitivity of ~86.67% and a specificity of 20% was obtained. This varies significantly from other studies conducted with rapid card tests for cardiac troponins in post mortem pericardial fluid. According to a study done by Zhipeng chao et. al, elevated levels of troponins have also been seen in cases such as hyperthermia, renal failure, cerebrovascular disease, drowning, carbon monoxide poisoning, as well as others, their study showed that statistically cTnT levels were higher in those with acute myocardial infarction.[4]

As such, elevated cardiac troponin levels solely cannot be relied upon to conform a diagnosis of AMI and needs to be substantiated with clinical/ histopathological evidence. Similarly, there may be cases in which all the evidence points towards Mi but without elevated troponin levels. The period between initiation of ischemia and time of death is also significant as it determines whether the troponins released from the cardiac myocytes reach the pericardial fluid after circulation and ultrafiltration of plasma or via direct diffusion into the pericardial fluid. However this data was difficult to retrieve due to the circumstances of the cases.

There are several contradictory findings regarding the positive correlation between detection of cardiac troponins in pericardial fluid, and incidence of MI. A majority of conducted related studies have reiterated the usefulness of troponin estimation in diagnosis of cardiac death, but are inconsistent with respect to the specificity and diagnostic value.[4,6–8,14] In view of these contradictory findings, this study was an effort to obtain a result in this regard, in our community of Victoria Hospital, a tertiary care centre.

Conclusion

After conducting this study, the following conclusions were reached:

- While estimation of troponin levels in pericardial fluid has a reliable sensitivity, its specificity is such that it cannot be used for the exclusive diagnosis of AMI. If used it must be supplemented by clinical and histopathological evidence.

Limitations of Study

- Inability to retrieve data on the time interval between initiation of ischemia and time of death.
- Lack of data on the histopathological findings of the myocardial tissue so as to confirm presence of infarction as biopsies could not be carried out for all cases.

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