Postmortem Cardiac Troponin-T levels in Heart Blood as a Marker to Diagnose Acute Myocardial Infarction

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Abstract

Sudden death accounts for approximately two-thirds of autopsies in Forensic Medicine. Natural death within 1 hour after the beginning of acute symptoms, is defined as Sudden Cardiac Death. Acute Myocardial infarction (AMI) is a serious and potentially lethal manifestation of coronary artery disease, affecting more than 7 million people worldwide each year and was proved to be a cause of sudden death. This study quantitatively analyzed the heart blood for Cardiac Troponin T (cTnT) levels within 48hours after death & correlated with the histological changes of myocardium.

Key words *: Acute Myocardial Infarction, Sudden Cardiac Death, Cardiac Troponin T,*

Introduction

The incidence of heart disease was less than 10% worldwide, at the starting of the 20th century. With the advent of 21st century, cardiac deaths accounted for 50% of all deaths in the developed world and one fourth in the developing world.[1]By 2020,heart disease will lead to 25 million deaths all over the world annually and coronary artery disease will take over infectious disease as the number one killer.[2]

One-6th of total populace lives in India and coronary disease represent 24% of all deaths. Among all the ethnic groups in the world, Indians have highest prevalence of coronary artery disease and the projected rise in the mortality rates is >100% in the next 25 years.[3]

In postmortem examination of dead bodies subjected to autopsy with probable sudden cardiac death, a diagnosis of myocardial infarction is usually made based on the finding of severe atherosclerotic occlusive coronary artery disease. The development of histological sequelae of the infarcted myocardium occurs only after significant time lag between onset of myocardial infarction and death.

Recently several studies have shown keen interest on the application of biomarkers to diagnose AMI. The inclusion of cardiac biomarkers like Troponin T enables to diagnose AMI in 1/3rd of patients who do not meet the criteria for diagnosis of myocardial infarction.

The prognostic value of Cardiac Troponin T is most important as it is not dependent on Age, Sex, ECG changes as well as levels of age old biochemical markers such as CKMB. Cardiac Troponin levels act as a specific and sensitive marker of cardiac cellular necrosis and myocardial infarction. The rise in Cardiac Troponin T levels occurs within 3-24 hours after AMI and persists up to 2 weeks in the living.[4]

In clinical practice, the cTnT assay has been approved for the diagnosis of AMI with high sensitivity and specificity. Few studies have been done to assess the use of serum cTnT as a biomarker in investigating coronary artery diseases.

The aim of this study is to estimate postmortem Cardiac Troponin T levels in blood to diagnose acute myocardial infarction in autopsy and to correlate Cardiac Troponin T levels with histopathological diagnosis of acute myocardial infarction in autopsy.

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Materials and Methods

This prospective study was conducted in the Mortuary of Chromepet Govt Hospital, Chennai and Department of Forensic Medicine, Sree Balaji Medical College, Chennai for a period of 10 months in 2019.

This study quantitatively analyzed the heart blood from the right and left ventricle and pericardial fluid for Cardiac Troponin T (cTnT) levels, in sudden deaths within 48hours after death, by a highly sensitive and rapid Cardiac Troponin T Electrochemiluminescence (ECL) immunoassay analyser and correlated with the histopathological findings of acute myocardial infarction. The study sample consisted of 12 hearts taken from cases of sudden deaths. A control sample (n = 6) consisting of deaths due to poisoning, and natural non cardiac causes like stroke, tuberculosis etc. were taken. Before getting into the study, Ethical clearance was obtained from the Institutional Ethical committee. (IEC)

In dead bodies with history of non traumatic sudden death subjected for autopsy, after making a longitudinal incision on the skin dissecting out the skin, subcutaneous tissue, fascia and muscle with a BP handle scalpel blade, reflecting out the skin together with the muscles, cutting the ribs at the costochondral junction along with the sternoclavicular joint disarticulating it and the sternum lifted up, dissected out exposing the thoracic cavity and the heart, blood was withdrawn from the pericardial cavity, right and left chamber of the heart with a 21G sterile syringe with needle and immediately transferred to a sterile sampling anticoagulant added test tube, corked with the stopper and sent for analysis at a private lab. Cardiac Troponin-T levels in heart blood serum was measured using Cobase411 immunoassay Analyser. T

The internal autopsy was followed by microscopic and toxicology examination. The cause of death was determined by correlating different histopathological features, with the levels of Cardiac Troponin T in heart blood.

Results

The study MI and control sample contained predominantly men (Table -1).While the average age of the control sample was 50(Table 2), the average age of the cardiac death sample was 52 (Table 2). In the control sample two patients died due to acute organophosphorus poisoning, three cases due to cerebrovascular accident/ metabolic encephalopathy, and one case due to pulmonary tuberculosis. Cardiac Troponin T level was markedly elevated **(>10ng/ml)** in all except one case of suspected MI in the study sample (Table -3) and also markedly elevated in the control sample except two cases (Table-4). Histopathological examination of the hematoxylin and eosin stained, heart slices, showed that four cases of the study group had evidence of early myocardial infarction with features of myonecrosis, waviness of fibers, nucleomegaly, pyknotic nuclei and polymorph infiltration (Table -5). Three cases of acute MI deaths demonstrated, myocardium with multiple foci of infarction showing hyalinised collagen fibers. In areas of old infarction, HPE revealed evidence of dense fibrous tissue replacing normal myocardial tissue. Four of the control cases showed normal histology of the myocardium, while two showed mild eosinophilia of the myocardial fibers with mild infiltration of polymorphs(Table -5).

Table: 1 Sex Distribution among the study sample and among the control group

	Study sample		Control sample	
Sex	**Frequency**	**Percentage**	**Frequency**	**Percentage**
Male	11	91.7%	5	83.3%
Female	1	8.3%	1	16.7%
Total	12	100%	6	100%

Table: 2 Age group wise distribution of the study sample and control sample group

Study sample			Control sample	
Age distribution	**No. of Cases**	**Percentage**	**No. of Cases**	**Percentage**
20-40 years	3	25%	1	16.6%
41-60 years	6	50%	4	67%
61- 80 years	3	25%	1	16.6%

Cardiac Troponin T concentration levels in heart blood and pericardial fluid by highly sensitive Cardiac Troponin T quantitative assay

Table 3: Study group (n=12)

S.No.	Cardiac Troponin T Levels (ng/ml)(> 0.1ng/ml +ve result)			Postmortem Interval	Inference
	Left Ventricle	Right Ventricle	Pericardial Fluid		
1.	>10	>10	>10	8 hours	Markedly increased
2.	>10	>10	>10	10 hours	Markedly increased
3.	>10	>10	>10	19 hours	Markedly increased
4.	>10	>10	>10	16 hours	Markedly increased
5.	>10	>10	>10	16 hours	Markedly increased
6.	>10	>10	>10	12hours	Markedly increased
7.	>10	>10	>10	17hours	Markedly increased
8.	>10	>10	>10	5 hours	Markedly increased
9.	>10	>10	>10	12hours	Markedly increased
10.	0.063	0.051	0.093	6 hours	Normal
11.	>10	>10	>10	18hours	Markedly increased
12.	>10	>10	>10	24 hours	Markedly increased

Table 4: Control group(n=6)

S.No.	Cardiac Troponin T Levels (ng/ml) (> 0.1ng/ml +ve result)			Postmortem Interval	Inference
	Left Ventricle	Right Ventricle	Pericardial Fluid		
1.	>10	>10	>10	30 hours	Markedly increased
2.	>10	>10	>10	28 hours	Markedly increased
3.	>10	>10	>10	40 hours	Markedly increased
4.	0.05	0.06	0.03	6 hours	Normal
5.	0.05	0.08	0.06	8 hours	Normal
6.	>10	>10	>10	36 hours	Markedly increased

Table 5: Histopathological examination results of study group & control group

S.No.	Observations	Study group No. of Cases	Control group No. of Cases
1	Evidence of early myocardial infarction	4	nil
2	Evidence of early myocardial infarction and old infarction	3	---
3	Evidence of old infarction	2	2
4	Normal myocardial histology. No areas of infarction	3	4

$$\text{Sensitivity} = \frac{\text{Total MI cases with positive cTnT test/HPE results}}{\text{Total of all MI cases tested.}}$$

Sensitivity = $11 \div 12 \times 100 =$ **91.66%**

$$\text{Specificity} = \frac{\text{Total non MI/healthy cases with negative cTnT test/HPE results}}{\text{Total of all healthy cases tested.}}$$

Specificity= $4 \div 6 \times 100 =$ **66.66%**

Discussion

WHO defines sudden death as those that occurs within 24 hours of onset of terminal illness. Coronary atherosclerosis contribute about 80 % of sudden cardiac deaths. Establishment of cause of death in cases of sudden cardiac death is a challenging task to the Forensic Pathologist. Any apparent gross change of infarction takes 24- 48 hours following occlusion of major coronary, resulting in autopsy identification of early myocardial infarction difficult. Additional insight may be obtained from history, environment and circumstance of death. Even though minimal microscopic evidence are recognized as early as 6 hours, in the absence of gross changes the involved area may be missed when random blocks are taken for histopathological examination . Studies on human heart during autopsy for detection of early myocardial infarction are scarce.

The basis of using the measurement of a protein in blood to detect cell straightforward and requires consideration of a few major factors:

Criteria for a blood marker of cell death

I. Sensitivity : Abundance in cell, Location in cell

II. Sample timing : Mode of entry into blood, Half-life of elimination

III. Specificity : Distribution in different cells or organs

The myocyte is the major cell in the myocardium, and the heart's action is to pump blood. Because myocytes essentially cannot be regenerated, if heart cells die, then cardiac function has a high probability of being impaired. When the cell dies, the proteins inside the cell will be released, with proteins in the cytoplasm leaving the cell more rapidly than ones in the membranes or fixed cell elements. The most sensitive markers should be those in highest abundance in the cell, and because the major function of the heart is contraction, the proteins involved in contraction and producing the energy to support it should be good candidates for biomarkers of cardiac injury which could be detected in blood. Also, one has to consider the means by which the markers can reach the blood. Since occlusion of blood flow is the primary cause of myocardial infarction, most of the proteins reach the blood via the lymphatics where they are prone for degradation leading to delayed appearance in the blood. Finally, specificity can be achieved if the protein has a cardiac specific form. While utilizing abundant cardiac proteins involved in contraction or energy production seems obvious, it is not exactly the way the field evolved. Cell injury markers can be identified via via gene expression or proteomics. However, these are recent advances which required sequencing entire genomes and the development of technologies which were not available until recently. Table 6 Characteristics of various studies involving Cardiac troponin T as a biomarker to diagnose Acute Myocardial Infarction

Cont... **Table 6 Characteristics of various studies involving Cardiac troponin T as a biomarker to diagnose Acute Myocardial Infarction**

Author	Age group	Postmortem Interval (PMI) (hours)	Analysed sample	Cardiac Troponin T levels
Ellingsen et al.5	4–92	3–75	Serum	Elevated (1.95microg/l)
Zhu et al. 6	0–94	< 48	Serum, Heart blood, Pericardial fluid	Markedly elevated (Pericardial fluid < Heart blood<Serum)
Khalifa et al.7	—	6–20	Serum	Elevated
Remmer et al.8	25–54	8–141	Pericardial fluid and serum	Elevated
González-Herrera et al.9	27–95	5–34	Pericardial fluid and serum	Markedly elevated
Carvajal-Zarrabal et al. 10	24–74	<8	Serum	Markedly elevated ($p<0.05$)

Similar to the above mentioned studies (Table 6), our study also showed markedly elevated Cardiac Troponin T levels in heart blood of right ventricle, left ventricle and pericardial fluid (>10ng/l) and was statistically significant.

In our study, two cases of heart blood, cTnT level from the control sample was within normal range for living patients, while the remaining samples showed high levels, maybe due to autolysis that would be false positive of AMI in a living patient. , others have reported levels similar to living persons and have dismissed the role of hemolysis and autolysis[11].Some have propounded that Cardiac Troponin T levels may be elevated in many deaths because of nonspecific cardiac injury due to lack of oxygen during the agonal period.

The aims of this short study was to ascertain the relationship of deaths due to acute MI between Cardiac Troponin T levels and correlate them with the HPE findings. Limitations of his study include factors like cohort size, autolysis, variation in time since death, and cold storage duration. Inspite of these shortcomings, the data was analysed that may help our understanding of Cardiac Troponin T.

Conclusion

This study showed that there is a strong relationship between postmortem Cardiac Troponin T (cTnT) reactivity with death caused by myocardial infarction correlating significantly with histopathology findings.

One issue with this study and studies upto date, is that Cardiac Troponin T levels are tested in obvious patients with acute MI and compared with patients who died of non cardiac causes. Inevitably, all of these studies concluded that increased Cardiac Troponin T levels correlate with the cause of death and supported the gross and HPE findings.

Ethical Clearance: obtained from Institutional Ethical Committee

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