



## CARDIAC TROPONIN T LEVEL IN SUDDEN DEATH CASES – A PROSPECTIVE STUDY

### Biochemistry

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### ABSTRACT

Troponin, or the troponin complex, is a complex of three regulatory proteins (troponin C, troponin I, and troponin T) that are integral to muscle contraction in skeletal muscle and cardiac muscle, but not smooth muscle. Measurements of cardiac-specific troponins I and T are extensively used as diagnostic and prognostic indicators in the management of myocardial infarction and acute coronary syndrome. Blood troponin levels may be used as a diagnostic marker for stroke, although the sensitivity of this measurement is low. In this prospective study, all the cases with history of sudden death that were autopsied from the date of 1st January 2019 to 31st December 2020 were analyzed at the department of Forensic Medicine and Biochemistry, Adichunchanagiri Institute of Medical Sciences. In the present study, Maximum number of victims (15 cases) belongs more than 50 years, with male female ratio is 2:1 and Maximum number of victims (20 cases) had serum Troponin T level >3000ng/ml.

### KEYWORDS

Cardiac Troponin T, Sudden Death

#### INTRODUCTION:

Troponin types comprises of 3 distinct protein types namely troponin C, troponin I, Troponin T and these protein units are essential for contractility in skeletal and cardiac muscle. In the management of Ischemic heart diseases, plasma level measurements of cardiac troponins I and T are widely employed as diagnostic and prognostic indicators. These troponin levels measurements in the blood may be an important early predictor of Stroke but sensitivity parameters of such measurements may be low.<sup>1</sup>

Troponin-tropomyosin complex is sandwiched between actin filaments in skeletal and cardiac muscle tissue. In a relaxed muscle state, tropomyosin inhibits contraction by blocking the myosin cross bridging. Whenever a muscle is activated to contract through an action potential impulse, calcium is released into sarcoplasm from the calcium channels. A portion of released calcium binds to troponin thereby enabling cross bridge formation between myosin and actin as a result of their binding. These events result in muscle contraction.

Whenever there is an ischemic injury to cardiac muscle, the cardiac specific troponins are released into blood and hence they serve as a very important marker for myocardium damage with a high degree of specificity and sensitivity. Their estimation in blood or plasma also assumes importance in diagnostic differentiation between myocardial infarction and unstable angina in people showing symptoms of chest pain or angina of acute coronary syndrome. Individuals suffering from recent Myocardial Infarction will have areas of ischemic or damaged area of myocardium resulting in subsequent release and raised cardiac specific troponins in the blood. Person's suffering from acute coronary insufficiency leading to Myocardial Infarction as a result of severe coronary vessels vasospasm can also have high levels of Troponins in their blood. Post-myocardial infarction, troponin levels may remain elevated for days to weeks.<sup>2,3</sup>

Cardiac specific troponins are regarded as distinct and specific biomarkers for all myocardial damage including myocardial infarction, a severe disease with high mortality. WHO has set a threshold target levels of 2 µg or more as MI diagnostic cutoff levels of raised Troponin levels in blood. These raised troponin levels should be taken into consideration along with deranged levels of many other cardiac biomarkers such as creatine kinase. Increased Troponin levels are also witnessed in conditions that affect the heart muscle and early death, such as Renal Failure, pancreatitis. Myocarditis etc.. Sometimes, various arrhythmias like supraventricular tachycardias, Ventricular fibrillation episodes may occur in Person's with normal

coronaries leading to raised levels of troponins in blood. This may be due to oxygen demand supply mismatch to the myocardium.<sup>4,5,6</sup>

Increased levels of Troponins are seen in variety of other cardiac disorders other than Ischemic heart diseases like heart failure, ventricular rhythm disorders, myocarditis, pericarditis, various types of cardiomyopathies or cardiac amyloidosis.

Traumatic causes of myocardial injury like cardiac contusion, Cardioversion including internal and external with defibrillation, can also increase troponin levels significantly. Other instances where Troponin levels are raised are any procedures involving cardiac surgery such as heart surgeries, heart Transplantation, ASD closures, percutaneous coronary intervention or radiofrequency ablation.

#### OBJECTIVE:

- This study is to estimation of cardiac Troponin T level in sudden death cases.

#### METHODOLOGY:

In this prospective study, all the cases with history of sudden death that were autopsied from the date of 1st January 2019 to 31st December 2020 were analyzed at the department of Forensic Medicine and Biochemistry, Adichunchanagiri Institute of Medical Sciences.

#### INCLUSION CRITERIA:

Autopsied cases with history of sudden death

#### EXCLUSION CRITERIA:

Autopsied cases where history unknown.

#### Procedure for Extraction of Biological Fluids:

During the autopsy, up to 6 mL of peripheral blood was collected for each patient from the femoral vein and pericardial sac, respectively, with a sterile syringe. The samples were allowed to clot and centrifuged within 2 h after extraction. Samples were stored for preservation at a temperature of 80C until the biochemical batch analysis was carried out.

#### Biochemical Determinations:

Ultra-sensitive troponin T (hs-cTnI) concentrations were determined using the "STAT High-Sensitive Troponin-T assay, which uses mouse monoclonal antibodies that recognize two epitopes in human hs-cTnI. It demonstrated a detection limit of 1.5 ng/L, a 99th percentile of <26 ng/L, and a coefficient of variation of 10% at 3 ng/L.

**STATISTICAL ANALYSIS:**

Data were presented either as mean  $\pm$  standard deviation (SD) or as percentage and median. A comparison between Groups A and B was made by Mann-Whitney U test/two sample *t*-tests for quantitative data and Fisher's exact test for categorical data. A factor influencing outcome of interest was considered significant if its *P* value was  $<0.05$ . Analyses were performed with the statistical software Strata, version 11.1.

**RESULTS:****Table 1: Year Wise Distribution Of Cases:**

| Year  | Total no. of poisoning cases |
|-------|------------------------------|
| 2019  | 08                           |
| 2020  | 12                           |
| Total | 20                           |

In 2019, total numbers of poisoning cases autopsied were 8 and in 2020 were 12.

**Table 2: Age & Sexwise Distribution Of Cases:**

| AGE GROUP     | MALE | FEMALE | TOTAL |
|---------------|------|--------|-------|
| < 20 YEAR     | -    | -      | -     |
| 21 TO 30 YEAR | -    | -      | -     |
| 31 TO 40 YEAR | 01   | -      | 01    |
| 41 TO 50 YEAR | 03   | 01     | 04    |
| >50 YEAR      | 08   | 07     | 15    |
| TOTAL         | 12   | 08     | 20    |

Maximum number of victims (15 cases) belongs more than 50 years, with male female ratio is 2:1.

**Table 3: Serum Troponin T Level:**

| PSEUDO CHOLINESTERASE LEVEL | NUMBER OF CASES |
|-----------------------------|-----------------|
| 1 to 0.4 ng/ml              | -               |
| >3000ng/ml                  | 20              |

Maximum number of victims (20 cases) had serum Troponin T level  $>3000$ ng/ml.

**DISCUSSION:**

In any autopsy diagnosis of Sudden or unexplained deaths including AMI, various biomarkers are implicated like cardiac troponins, LDH, BNP, NTproBNP, CK-mB etc. However, these markers sometimes are in higher concentrations when measured earlier in the pericardial fluid compared to peripheral blood sample, maybe due to their proximity to myocardium or rather a passive diffusion seen in early postmortem phase. There was an effort to narrow down the search to accuracy and ideal biomarkers reflecting the cardiac injury. Then evolved the hypothesis that estimation of ratio between cTnI values derived in pericardial fluid and that of peripheral blood may give an useful insight in the autopsy diagnosis of AMI deaths. In the current study, it was observed that maximum number of victims (15 cases) belonged to 50 years and above with male female ratio being 2:1 and Maximum number of victims (20 cases) had serum Troponin T level  $>3000$ ng/ml. Observations from a study done by Diana HR, revealed that out of 80 identified cases in the average age group  $44.5 \pm 19$  years, there was a significant correlation of Hs-TnT levels in pericardial fluid and heart blood between cardiac and non-cardiac groups with a *p*-value of 0.14 and 0.04 respectively. Pericardial fluid analysis had superior sensitivity (75%) and specificity (64%) with a cutoff level at 17.72 ng/ml and an area under the curve at 0.747, when Receiver-operator characteristic curves analysis was applied for study. The study also highlighted a significant correlation between postmortem interval and Hs-TnT levels estimated in pericardial fluid, cardiac and peripheral blood. The conclusion was that estimation of cardiac troponin T levels using a very sensitive assay in pericardial fluid may be an important guide and tool in autopsy diagnosis of sudden cardiac death.<sup>7</sup>

Analyzing the study done by Zhipnego, a meta-analysis study revealed that postmortem cTn I and cTn T levels were significantly raised in pericardial fluid and blood in cardiac deaths, including patients suffering from acute myocardial infarction (AMI). The determined postmortem cut-off values of cTnI was 86.2ng/mL in the pericardial fluid and 9.5mg/mL in the Serum whereas serum cTnT levels were 8.025 ng/mL.<sup>8</sup>

In a study done by Omalu, a chemiluminescent microparticle immunoassay system was employed to assay the titers of troponins.

The postmortem cTnI plasma levels at autopsy performed within 24 hours of death in every decedent who died from MI type 3 were estimated and documented. When a period of 2 years was identified, it was confirmed from autopsy that 52 decedents dying due to MI type 3 had a coronary atherosclerotic disease. Persons dying from sudden death complicated by MI were in the age group between 40 to 78 yrs and the mean estimated age was 60.6 years. Among them, Majority 38 (73%) were men and 14 (27%) were women. Postmortem cTnI plasma levels were within the normal reference range (0.01-0.30 ng/mL) in ten percent of decedents studied, while the rest majority of the decedents showed raised cTnI plasma levels during autopsy, ranging from 0.31 to values exceeding 4400 ng/mL. Double or triple vessel luminal coronary occlusion with significant severity of stenosis (75% to 100%) was witnessed in 69% of the decedents.<sup>9</sup>

An interesting study done by Lai PS using comparatively metrics of ante-mortem and postmortem subjects with certified MI was analyzed. Here, 38 MI confirmed or certified cases presenting to emergency were compared with 8 postmortem certified cases of MI. The observed mean values for post-mortem (PM) cases ( $5.065 \pm 4.498$  ng/ml) exceeded by a fair value in ante-mortem (AM) cases ( $1.327 \pm 1.918$  ng/ml). This shows a fair statistical significance in the differences between the ante-mortem and post-mortem MI cases studied. So, it could be shown that post-mortem Trop T level is ideally not taken into consideration to reflect the ante-mortem Trop T level in the diagnosis of myocardial infarction. Also, additionally, the analysis or performing interval of Troponins levels in postmortem period varies in different conditions and this effect of post-mortem performing interval assumes statistical significance and ranges between interval of  $< 12$  hours and 12 - 48 hours.<sup>10</sup>

**CONCLUSION:**

In summary, the study highlighted the fact that postmortem measurements of specific cardiac biomarkers were significantly helpful in diagnosis of sudden deaths, unexplained deaths where cause of death is difficult to establish. However, more frequently, only the gross or pathological analysis is not conclusively helpful requiring additional complementary tests to arrive at a diagnosis. Hence, the postmortem measurements of the biomarkers which carry a very high specificity and sensitivity, becomes a highly effective and vital tool for use in Forensic field that comes in the aid of diagnosis in sudden natural or unexplained deaths where the autopsy findings remain inconclusive.

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