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Original communication

The effect of elapsed time on cardiac troponin-T (cTnT) degradation and its dependency on the cause of death

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ABSTRACT

Purpose: The aim of the present study is to evaluate the effect of elapsed time on cardiac troponin-T degradation and its dependency on the cause of death.
Methods: The cases included in this study were divided into six groups depending upon the cause of death without any prior history of disease that died in the hospital and their exact time of death was known. The analysis involves extraction of the protein, separation by denaturing gel electrophoresis and visualization by Western blot.
Results: Western blot data shows the rate of degradation of cTnT into lower molecular weight fragments with respect to time. In cases of control group the greatest amount of protein breakdown was observed within the first 64 h while in MI cases within first 6 h, the original band of cTnT (42 kDa) decreased markedly into seven major fragments, with 25 kDa & 20 kDa fragments being the most prominent. In burn group, at 41.40 h blot shows maximum fragmentation. In electrocution group the greatest amount of protein breakdown was observed within the first 50 Hrs. Within asphyxia cases, the original band of cTnT (42 kDa) decreased markedly into many major and minor fragments which continues up to 210 Hrs while the original band of cTnT (42 kDa) in poisoning cases decreased markedly into many major & minor fragments up to 140 h but after it blot shows only intact protein of very less intensity with few minor fragments.
Conclusion: It can be observed that in case of death due to MI, the intact cTnI fragmented at a much faster rate than in burn, electrocution, control, poisoning and asphyxia group. Thus, the rate of fragmentation of intact cTnT into lower molecular weight fragments depends upon the cause of death.

1. Introduction

In developing countries population explosion, poverty and increasing stress and strain in daily life, often lead to cases of suicides, homicides and accidents. With urbanization, rural areas are also not left aloof and this can be seen from the increasing incidence of suicides as well as of homicides.[1–3] It's extremely important to study PMI in different causes of death since it assists in a great way in making an opinion on the exact cause of death following such incident often times. In forensic practice, is not unavoidable for a crime to be masked by the suspect. The accused might have set the scene. With diligent knowledge of the interval one could really say as an expert that the cause of death is not feigned hence there is a great need in evaluating such death to have been at the crime scene before performing an autopsy on such body. A qualitative approach was followed in order to see whether the degradation of cTnT is dependent upon the cause of death.

The cardiac troponin [cTn] has been known as a marker of heart damage and myocardial cell death for more than 10 years. In toxicological studies Troponin has been established as a biomarker for drug-induced cardiac injury. Previous studies have suggested the possible application of cTnT in the postmortem diagnosis of acute myocardial infarction, cardiac contusions and for the estimation of postmortem interval.[4–6] But due to the lack of data and standardized procedures a general agreement has not been established to use cardiac troponin in forensic casework. But, since heart

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is a well protected organ, the effect of external conditions is less as compared to the skeletal muscles and this protein is an excellent substrate for proteases. The proteolytic degradation of cTnT is the result of its primary amino acid sequence, which is rich in sites that serve as a substrate for protease. Therefore, cTnT was chosen as the material of study. It undergoes degradation with time which proceeds in an orderly manner leading to the appearance of a wide diversity of short fragments of it. This disappearance of intact cardiac troponin into lower molecular weight fragments can be easily monitored by western blotting.

Cardiac troponin T is a 37 KD protein that is [10–30] % dissimilar from skeletal Troponin T isoforms.[7] Troponin T [TnT] binds to tropomyosin [Tm] to anchor the troponin complex in the thin filament and it thus serves as a vital link in the Ca^{2+} regulation of striated muscle contraction. The enzyme linked immunoassay was developed for cTnT and showed its potential role towards cardiac myocytes as an AMI marker in 1989 and 1991 by Katus and Gerhardt respectively.[8,9] Proteases such as calpains, cathespins and serine proteases have showed active role in degradation of cTnT.[10,11] It has been shown that the calcium activated cysteine proteases such as μ-calpain [calpain1] and caspase-3 are capable of degrading cTnT [and cTnI] in vitro. Studies in the rat and mouse have shown that μ-calpain activation results in a 27 kDa fragment after ex-vivo ischemia and reperfusion of the isolated heart. Furthermore, addition of inhibitors of μ-calpain showed a decrease in degradation of cTnT.[11]

The present study aimed to evaluate the effect of elapsed time on cardiac troponin-T protein degradation and its dependency on the cause of death [Poisoning, Asphyxia, Burn, Electrocution MI and Control].

2. Materials and methods

Cardiac tissue samples were collected from medico-legal autopsies, [department of Forensic Medicine and Toxicology], King George's Medical University [K.G.M.U.], Lucknow India, after informed consent from the relatives and studied post-mortem degradation by incubation of the cardiac tissue at room temperature [RT] for different time periods. A prior approval was obtained from the K.G.M.U. ethics committee vide letter no-865/R-Cell-12. Ref. code: 55 E.C.M.II A/P20 to conduct this research. The cases included in this study were divided into six groups depending upon the cause of death-Group 1 [Control, n = 10], Group 2 [Asphyxia, n = 10], Group 3 [Poisoning, n = 10], Group 4 [Burn, n = 10], Group 5 [Electrocution, n = 10] and Group 5 [Myocardial Infarction [MI], n = 10] without any prior history of disease that died in the hospital and their exact time of death were known. The experimental design for this work is summarized in Fig. 1.

3. Tissue homogenization

Tissue homogenization was done by taking 1 g of cardiac tissue sample with 4 ml extraction buffer consisting of 25 mM Acetic acid/Acetate in 6 M Urea, pH 4.6, using 6 M NaoH or 6 M HCL and one ml of the EZBlock™ protease inhibitor cocktail, EDTA-Free [K272-1 ML, BioVision]. The samples are then centrifuged at 5000 g for 5 min. The resulting supernatant was aliquoted and stored at −80 °C until used.

3.1. Protein quantification

An aliquot from each tissue sample was thawed and the protein content was quantified using the ELITech Clinical Systems with Biuret End point method which is based on the principle, proteins form a colored complex in the presence of copper salt in alkaline solution.

3.2. Gel preparation [12% SDS-PAGE GEL]

Mix all components of Running [Bottom] Gel in that order and promptly pipette into assembled gel plates evenly from side to side [dH_2O, 1.5 M Tris [pH 8.8], 10% SDS, acrylamide:bisacrylamide ratio of 29:1, 10% APS, TEMED]. Add a small layer of water-saturated butanol in order to produce a clean, straight top of the running

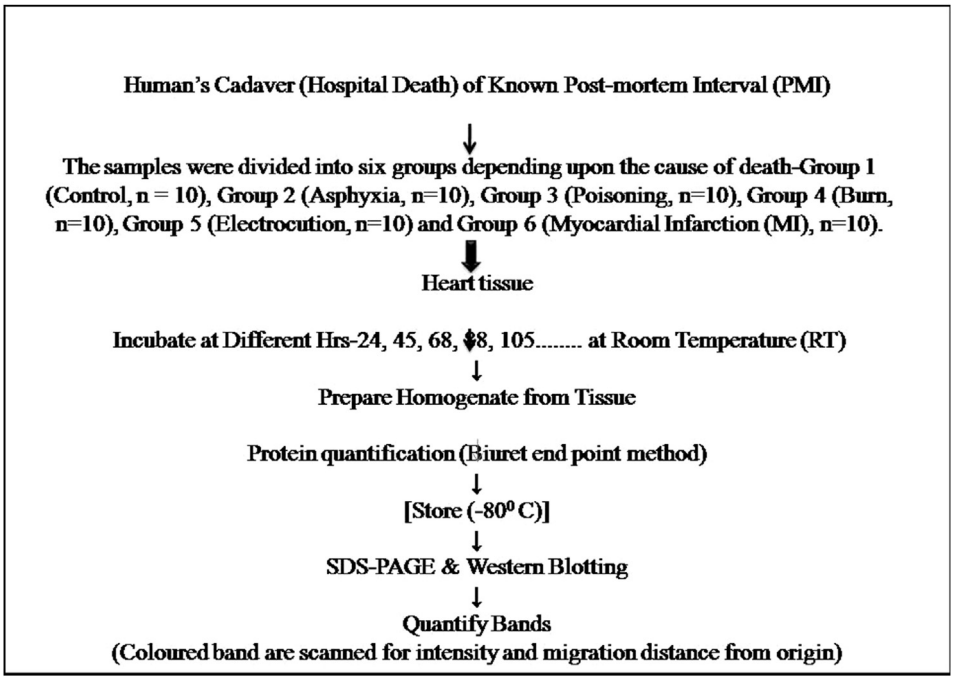

Fig. 1. A flow chart of the experimental design used in this study.