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Temperature-Dependent Post-Mortem Changes in Human Cardiac Troponin-T (cTnT): An Approach in Determining Postmortem Interval

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Abstract : Globally approximately 55.3 million people die each year. In the India there were 95 lakh annual deaths in 2013. The number of deaths resulted from homicides, suicides and unintentional injuries in the same period was about 5.7 lakh. The ever-increasing crime rate necessitated the development of methods for determining time since death. An erroneous time of death window can lead investigators down the wrong path or possibly focus a case on an innocent suspect. In this regard a research was carried out by analyzing the temperature dependent degradation of a Cardiac Troponin-T protein (cTnT) in the myocardium postmortem as a marker for time since death. Cardiac tissue samples were collected from (n=6) medico-legal autopsies, (in the Department of Forensic Medicine and Toxicology, King George's Medical University, Lucknow India) after informed consent from the relatives and studied post-mortem degradation by incubation of the cardiac tissue at room temperature (20±2 OC), 12 0C, 25 0C and 37 0C for different time periods ((~5, 26, 50, 84, 132, 157, 180, 205, and 230 hours). The cases included were the subjects of road traffic accidents (RTA) without any prior history of disease who died in the hospital and their exact time of death was known. The analysis involved extraction of the protein, separation by denaturing gel electrophoresis (SDS-PAGE) and visualization by Western blot using cTnT specific monoclonal antibodies. The area of the bands within a lane was quantified by scanning and digitizing the image using Gel Doc. The data shows a distinct temporal profile corresponding to the degradation of cTnT by proteases found in cardiac muscle. The disappearance of intact cTnT and the appearance of lower molecular weight bands are easily observed. Western blot data clearly showed the intact protein at 42 kDa, two major (27 kDa, 10kDa) fragments, two additional minor fragments (32 kDa) and formation of low molecular weight fragments as time increases. At 12 0C the intensity of band (intact cTnT) decreased steadily as compared to RT, 25 0C and 37 0C. Overall, both PMI and temperature had a statistically significant effect where the greatest amount of protein breakdown was observed within the first 38 h and at the highest temperature, 37 0C. The combination of high temperature (37 0C) and long Postmortem interval (105.15 hrs) had the most drastic effect on the breakdown of cTnT. If the percent intact cTnT is calculated from the total area integrated within a Western blot lane, then the percent intact cTnT shows a pseudo-first order relationship when plotted against the log of the time postmortem. These plots show a good coefficient of correlation of r = 0.95 (p=0.003) for the regression of the human heart at different temperature conditions. The data presented demonstrates that this technique can provide an extended time range during which Postmortem interval can be more accurately estimated.

Keywords : degradation, postmortem interval, proteolysis, temperature, troponin
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