Original Research

Role of Cardiac Troponin I in Case of Death Time Estimation- An in Vitro Study

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Abstract

Background- Beta blockers are used for treating hypertension but few of them when taken in overdose can be fatal. Acebutolol is one such beta-adrenoreceptor blocking agent that is considered one of the most toxic beta blockers when taking overdose. Determination of postmortem interval is a common practice in medicolegal investigation.

Objective: The present study was conducted to investigate the degradation pattern of cTnI with respect to time.

Materials And Methods: Group 1 were anesthetized with Sodium pentobarbital (40 IP). The chest cavity was then quickly opened, and the heart was excised out. Group 2 (Asphxial Death) were sacrificed by clamping a tracheostomy tube. Group 3 (Acebutolol Death) were sacrificed by giving an oral lethal dose of Acebutolol(6620mg/kg). Incubation at 28º C for different time interval (0 hrs, 6 hrs, 12 hrs, 24hrs, 48 hrs, 72 hrs, 96 hrs and 100 hrs). The heart was then isolated and freezed at-80ºC.The extraction of protein from tissue was optimized so as to stop or minimize the degradation of protein during the extraction process. The conventional method was used for extraction with slight modifications in the extraction buffer and an added step of carrying every step of the extraction process at low temperature (1-4ºC)

Results- The data clearly established that the degradation pattern of cTnI is different for all the three groups- Control, Acebutolol and Asphyxia. In case of Acebutolol the degradation of cTnI into its molecular fragments is at a much faster rate when compared to the Control and Asphyxia groups, The difference in degradation of cTnI is because Acebutolol is highly cardiotoxic, and its effect on cardiac tissues is high and immediate.

Conclusion- The degradation of intact cTnI into smaller molecular fragments is also dependent upon temperature. In order to know more about the measurement of cTnI fragmentation for the determination of the postmortem interval, further investigations are necessary to understand more about the cTnI degradation pattern.

Keywords- *Acebutolol, Cardiotoxicity, Asphyxia, Beta-Adrenoreceptor Blocking, Cardiac Troponin (CTn)*

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Introduction

In developing countries, population explosion, poverty, 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] A large number of suicides are encountered due to asphyxia death. The word Asphyxia was derived from the Greek and means 'pulseless'. It is a condition of the severely deficient supply of oxygen to the body that arises from being unable to breathe normally. When a person is subjected to the asphyxia, unconsciousness generally occurs in 2 - 3 mins, and death in 4 5 mins.[2,3] However, it is now recognized that these are in no ways sensitive or specific for asphyxia. Thus, as there are no diagnostic pathological features of acute asphyxia, autopsies may have negative or non-specific findings that might only suggest the possibility.

Beta blockers are used for treating hypertension but few of them when taken in overdose can be fatal. Acebutolol is one such beta-adrenoreceptor blocking agent that is considered one of the most toxic beta blockers when taking overdose.[2,3]

The cardiac troponin (cTn) has been known as a marker of heart damage and myocardial cell death for more than 10 years.[3] In toxicological studies Troponin has been established as a biomarker for drug-induced cardiac injury. Previous studies have suggested the possible application of cTnI in the post-mortem diagnosis of acute myocardial infarction, cardiac contusions and for the estimation of post-mortem interval.[4-9] TnI is present both in skeletal as well as cardiac muscles. But, since heart is a well-protected organ, the effect of external conditions is less as compared to the skeletal muscles. Therefore, cTnI 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. But due to the lack of data and standardized procedures a general agreement has not been established to use cardiac troponin in forensic case work. Techniques based on biochemical changes offers advantage over physical methods since they are neither altered nor contaminated easily and rapidly.

So, the present study was conducted to investigate the degradation pattern of cTnI with respect to time. An analysis of cardiac troponin I with regard to the cause of death (Cardio toxicity due to Acebutolol, Asphyxia, and control) was studied.

Materials and Methods

This was a comparative analytical trial conducted on total 33 cases, in the time period of 4 months from August 2019 to December 2019.

Subjects were divided into three groups, Group 1 (Control, n = 11), Group 2 (Asphyxia, n=11), Group 3 (Acebutolol, n=11). The technique involved in the study for the detection was Western blot, which could be a technique to detect the expression of a selected protein in tissue homogenates.

Chemicals used in the study for sample buffer- 0.125 MTrisHCl (pH6.8), 4% (w/v) SDS, 20% (v/v) Glycerol, 10 % (v/v) β Mercaptoethanol, 0.01% (w/v) acid-base indicator, for running buffer- 250 mMTris, 1.92M Glycine, 1% SDS, for polyacrylamide gel Solution- 30% Acrylamide, 10% SDS, 10% APS, TEMED, 1.5MTris (pH 8.8), H2O. Armamentarium used in the study included Homogenizer: RQ127 (Remi Motors, India), Ultra High Speed Centrifugation machine: Remi Motors, India, Blotting Kit: C.B.S. Scientific Company, 300VPowerPac: C.B.S. Scientific Company.

Group 1 were anesthetized with Sodium pentobarbital (40 IP). The chest cavity was then quickly opened, and the heart was excised out. Group 2 (Asphyxial Death) were sacrificed by clamping a tracheostomy tube. Group 3 (Acebutolol Death) were sacrificed by giving an oral lethal dose of Acebutolol (6620 mg/kg). Incubation at 28º C for different time interval (0 hrs, 6 hrs, 12 hrs, 24hrs, 48 hrs, 72 hrs, 96 hrs and 100 hrs). The heart was then isolated and freezed at-80ºC.The extraction of protein from tissue was optimized so as to stop or minimize the degradation of protein during the extraction process. The conventional method was used for extraction with slight modifications in the extraction buffer and an added step of carrying every step of the extraction process at low temperature (1-4ºC). Frozen myocardial samples were cut into small pieces in sample trays placed on dry ice. 1 g of cardiac tissue was homogenized with 5 ml of ice-cold extraction buffer (10 mm sodium phosphate, 100 mm Tries, 200 mm Sodium chloride, 0.1% sodium azide, 1 tablet in 50 ml of Complete Protease Inhibitor cocktail, ph8.0). Samples are then centrifuged at 5000 g for 10 min. The supernatant was collected for analysis. The proteins migrate in response to an electrical field through pores in the gel matrix. The combination of gel

pore size and protein charge, size and shape determines the migration rate of a protein. Proteins separated using SDS-PAGE before are transferred to a membrane where they're probed by antibodies specific to the target protein Following a blocking step to stop any nonspecific binding of antibodies to the surface of the membrane, the membrane is probed with a primary antibody that attaches to the antigen of interest. After a washing step, the membrane is incubated with a secondary antibody that's reactive toward the first antibody. After probing with secondary antibody, the membrane is washed again and incubated with an appropriate enzyme substrate. one amongst the substrates used for protein detection is luminol-based and produce a chemiluminescent signal. Chemiluminescence could be a reaction that produces energy released within the kind of light within the presence of peroxidase and a peroxide buffer. The intensity of the signal after development should correlate with the abundance of the antigen on the membrane. Ethical clearance was obtained from the moral committee of the institute. Statistical analysis was done by Oneway ANOVA on the three groups.

Results

The data clearly established that the degradation pattern of cTnI is different for all the three groups- Control, Acebutolol and Asphyxia. In case of Acebutolol the degradation of cTnI into its molecular fragments is at a much faster rate when compared to the Control and Asphyxia groups, The difference in degradation of cTnI is because Acebutolol is highly cardiotoxic, and its effect on cardiac tissues is high and immediate. The myocardial damage is maximum in case of Acebutolol poisoning and then in case of Asphyxia and then in Control group. The two groups were compared with the control group. It was observed that in case of death due to Acebutolol poisoning the rate at which intact cTnI fragmented into lower molecular weight fragments was high as compared to that of control and asphyxial group (TABLE-1)

A one-way ANOVA was used to test for preference differences among the three groups. The null hypothesis was that no difference exists between the populations. The molecular weights differed significantly across the three. The F value is greater than the F critical value and thus the null hypothesis (H0) is rejected (TABLE-2)

TABLE 1: COMPARISON OF DECREASE IN MOLECULAR WEIGHTS OF cTnI WITH RESPECT TO TIME IN VARIOUS GROUPS OF STUDY

HOURS	CONTROL	ACEBUTOLOL	ASPHYXIA
0	23	24	23
6	23	15.4	21.4
12	22.9	13.4	19.1
24	21.8	12.8	17.1
48	16.9	9.8	15.4
72	14.8	7.5	12.4
96	7.16	6.2	7.11

TABLE 2: STATISTICAL ANALYSIS OF CHANGE IN MOLECULAR WEIGHTS WITH RESPECT TO POSTMORTEM INTERVAL IN THE THREE GROUPS OF STUDY

GROUPS	COUNT	SUM	AVERAGE	VARIANCE
Control	8	132	18.57122868	38.62914
Acebutolol	8	90.8	12.82837152	40.04904
Asphyxia	8	116.6	16.6	35.14

Cont... **TABLE 2: STATISTICAL ANALYSIS OF CHANGE IN MOLECULAR WEIGHTS WITH RESPECT TO POSTMORTEM INTERVAL IN THE THREE GROUPS OF STUDY**

		ANOVA			
Source of Variation	SS	do	F	P-value	F crit
Between Groups	122.0781953	260.5380464	1.49473	0.220320	3.454456
Within Groups	683.168471 6	16	37.95834506		
Total	804.345665 5	22			

Discussion

A qualitative approach was followed to determine whether the degradation of cTnI relies upon the rationale for death. Rate of degradation of cTnI into lower mass fragments with time within the three different cases and was observed that just just in case of death thanks to Acebutolol poisoning, the intact cTnI fragmented at a far faster rate than up to hurry and Asphyxia group. Thus, the speed of fragmentation of intact cTnI into lower mass fragments depended upon the rationale for death. An increasing death rate as results of violence constitutes an oversized group in medico legal autopsies. During the 21-year period, there have been 134 asphyxial deaths autopsied by the Department of medication which comprise 15.7% of all forensic autopsies; with 20.8% of the cases are aged between 30 and 39 years. Although it absolutely was varying per the methods of asphyxiation, suicide was found to be the design of death within the bulk of the cases.[10] during a study, the peripheral levels of cardiac troponin T (cTnT) in serial medico legal autopsy cases with a survival time <24 h and within 48 h post-mortem to assess the validity of investigating myocardial damage with special relevance traumatic causes of death. These included blunt and sharp instrument injury (n=122 and 21, respectively), asphyxiation (n=35), drowning (n=27), fire fatalities (n=94), hyperthermia (n=13), hypothermia (n=6), fatal methamphetamine (MA) abuse (n=12) and monoxide gas (CO) poisoning (n=5) as compared with infarction (MI, n=57) and cerebrovascular diseases (n=13). Cases within 12 h post-mortem usually showed lower cardiac and pericardial cTnT levels than did those of longer post-mortem time of 12–48 h. within the first post-mortem period of <12 h, significantly elevated serum cTnT levels were observed for hyperthermia.7 just in case of Control group the foremost bands of size 23, 23, 22.9, 21.8, 16.9, 14.8 and 7.16 kDa were observed at 0, 6, 12, 24, 48, 72 and 96 hrs respectively. just in case of Acebutolol group the foremost bands of size 24, 15.9, 13.4, 12.8, 9.8, 7.5 and 6.2 kDa were observed at 0, 6, 12, 24, 48, 72 and 96 hrs respectively. Just in case of Asphyxia group the foremost bands were of size 23, 21.4, 19.1, 17.1, 15.4, 12.4 and 7.11 kDa were observed at 0, 6, 12, 24, 48, 72 and 96 hrs respectively. Cardiac troponin T (cTnT) and troponin I (cTnI) became acknowledged as useful biochemical markers of drug-induced cardio toxicity. During this study we examined the discharge kinetics of cTnT and cTnI using an in vitro model of isolated rat neonatal ventricular cardiomyocytes (NVCM, 72h treatment with 0.1-3 microM of daunorubicin) and compared it with data from a rabbit model of chronic anthracycline-induced cardiomyopathy in vivo (3mg/kg of daunorubicin weekly, 10 weeks). In cell-culture media, the cTnI and cTnT concentrations were concentration- and time-dependently increasing in response to daunorubicin exposure and were negatively exponentially related to cardiomyocyte viability. With 3microM daunorubicin, the relative increase of AUC of cTnT and cTnI was 2.4- and 5.3-fold over the increase of LDH activity, respectively. In rabbits, the daunorubicin-induced cardiomyopathy was associated with progressive increase of both cTnT and cTnI. Although the correlation between cTnT and cTnI cumulative release (AUCs) was found (R=0.81; P<0.01) and both cardiac troponins corresponded well with the echocardiographically-assessed systolic dysfunction (R=0.83 and 0.81 for cTnT and cTnI, respectively; P<0.001), the first significant increase in cTnI levels was observed earlier (at a cumulative daunorubicin dose of 200mg/m(2)) than with

cTnT (350mg/m(2)). finally, our study has confirmed cTnT and cTnI as very sensitive and specific markers of anthracycline-induced cardiotoxicity.[8] during a study we examined the discharge kinetics of cTnT and cTnI using an in vitro model of isolated rat neonatal ventricular cardiomyocytes (NVCM, 72h treatment with 0.1-3 microM of daunorubicin) and compared it with data from a rabbit model of chronic anthracycline-induced cardiomyopathy in vivo (3mg/kg of daunorubicin weekly, 10 weeks). In cell-culture media, the cTnI and cTnT concentrations were concentration- and time-dependently increasing in response to daunorubicin exposure and were negatively exponentially related to cardiomyocyte viability. With 3microM daunorubicin, the relative increase of AUC of cTnT and cTnI was 2.4- and 5.3- fold over the increase of LDH activity; respectively.[6] The info clearly established that the degradation pattern of cTnI is different for all the three groups- Control, Acebutolol and the Asphyxia. Just in case of Acebutolol the degradation of cTnI into its molecular fragments is at a far faster rate when put next to the Control and Asphyxia groups. The difference in degradation of cTnI is because Acebutolol is extremely cardiotoxic, and its effect on cardiac tissues is high and immediate. The myocardial damage is maximum {in case just in case} of Acebutolol poisoning then in case of Asphyxia then up to hurry group. The degradation of cTnI relies upon the severity of myocardial damage at the time of death. The 2 groups were compared with the control group. it absolutely was observed that just in case of death thanks to Acebutolol poisoning the speed at which intact cTnI fragmented into lower mass fragments was high as compared thereto of control and asphyxial group. A one-way ANOVA was accustomed test for preference differences among the three groups. The null hypothesis was that no difference exists between the populations.

Conclusion

The degradation pattern of cardiac troponin I can be used for determining post-mortem interval and its rate of degradation is dependent upon the cause of death which can be used for establishing the cause of death. It was also evident in our study that the degradation of intact cTnI into smaller molecular fragments is also dependent upon temperature. In order to know more about the measurement of cTnI fragmentation for the determination of the post-mortem interval, further investigations are necessary to understand more about the cTnI degradation pattern.

Conflicts of Interest: The author declare that there is no conflict of interest regarding the publication of this paper.

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