



## STUDY OF PERICARDIAL FLUID BIOCHEMISTRY FOR ESTIMATION OF TIME SINCE DEATH

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**ABSTRACT** The estimation of time since death at the time of autopsy has been and remains to be one of the challenges to the Forensic Pathologist. A total of 100 study cases were randomly selected after screening. The pericardial fluid was examined biochemically for enzyme activity of Amylase, Creatine Kinase (CK), Gamma-glutamyl Transferase (GGT) and Lactate Dehydrogenase (LDH) enzymes by photoelectric colorimetry method. The enzyme activity levels so obtained were charted and statistically studied and graphical records obtained against known post-mortem interval. The data thus obtained was analysed with a view to ascertain whether such assays could be of any help to estimate time since death routinely. In this study we observed a positive correlation of all the four enzymes with the time elapsed after death of which rise in CK was found to be statistically significant.

**KEYWORDS :** Post-mortem interval (Time since death), Pericardial fluid, Enzyme activity, Amylase, Creatine Kinase (CK), Gamma-glutamyl transferase (GGT), Lactate Dehydrogenase (LDH)

## INTRODUCTION

In today's scenario, medico-legal experts routinely rely solely on age-old subjective methods of observing the external as well as the visceral somatic changes in the dead body that take place after death like cooling of body, changes in the eye, rigor mortis, hypostasis, signs of decomposition, mummification, adipocere formation, maggot infestation etc and the circumstantial evidences for estimation of time since death. No objective method is available which is accurate, reproducible and unequivocally accepted; hence, there is a need to re-explore objective methods which would be reproducible and accurate such as biochemical assays etc.

This study was an effort to find out whether it is practical and significant enough to estimate post-mortem interval by analyzing quantitatively the pericardial fluid enzyme activity changes. As a dead body is a biological material, there would be inherent biological variations in ante mortem and post-mortem enzyme activity in pericardial fluid.

## MATERIAL AND METHOD

A total of 100 cadavers brought to the Department of Forensic Medicine for medico-legal autopsy were studied. The cases with known time of trauma, known time of death and known mode of death were selected. As Pericardial fluid is known to be the ultra filtrate of blood the cases in which pre-mortem disturbance of body fluids, blood electrolytes and enzymes were suspected were excluded i.e. cases of poisoning, burn, septicemia, sudden death, subjects brought dead to emergency with unknown history, history of alcohol intake prior to death, chronic alcoholic, vomiting, diarrhoea, blood transfusion, diuretic therapy, heart disease, liver disease, kidney disease, pancreatic disease, epilepsy, myopathy, chronic drug intake, chronic smoker. A valid informed consent from the legal heirs of the deceased was taken before collection of pericardial fluid sample. 5ml pericardial fluid was collected in a sterile syringe from the posterior sinus or the pericardial sac and transferred to a clean vial. Samples found contaminated with blood were discarded. The collected sample was immediately centrifuged at 3000 rpm for 10 minutes and supernatant fluid was analysed for enzyme activity by kit method using semiautomatic analyzer by standard protocols (Amylase by direct substrate method, CK by modified IFCC method, LDH by modified IFCC method and GGT by carboxy substrate method) using commercial kit (Crest Biosystems). The data thus collected was analysed specifically for relation between pericardial fluid enzymes activities and time since death.

## RESULTS

Total 100 cases were studied of age range 8-70 years with maximum

cases belonging to 20-30 year age group (66 cases). The manner of injury sustained was wide- ranging from Road traffic accident (43 cases), Fall from height (6 cases), Poisoning (12), Burn (11), Stab/cut throat (7), Firearm/electrocution (8), Asphyxia (6), Natural causes (7). We compared mean of enzyme activity of all four enzymes (Amylase, CK, GGT and LDH) with time passed since death and statistical significance of enzyme activity changes between three ranges of time since death (<6 hrs, 6-12 hrs and 12-24 hrs). The observations made were as follows:-

### Amylase:

Mean of Amylase activity decreased between group of <6 hr and 6 to 12 hr and then increased in 12 to 24 hr group. On comparison between < 6 hr and 6 to 12 hr groups the decrease was not significant (p value > 0.05), however a rise in enzyme activity was noted on comparison between < 6 hr and 12 to 24 hr groups and between 6 to 12 hr and 12 to 24 hr groups though the rise was statistically not significant (p value > 0.05). A positive correlation was noted between Amylase activity and time since death (r-value +0.277) but it was statistically in-significant.

### Creatine Kinase (CK):

Mean value of Creatine Kinase (CK) activity increased between groups of <6 hr and 6 to 12 hr and also between 6 to <12 hr and 12 to 24 hr groups. When we compared CK levels between < 6 hr and 6 to 12 hr groups the increase was not significant (p value > 0.05) but interestingly on comparing between < 6 hr and 12 to 24 hr groups a rise in enzyme activity was noted which was statistically highly significant (p value < 0.01). On comparison between 6 to 12 hr and 12 to 24 hr groups a rise was seen but was statistically not significant (p value > 0.05). A positive correlation was noted between CK activity and time since death (r-value +0.325) which was statistically significant (p value < 0.05).

### Gamma-Glutamyl Transferase (GGT):

Mean value of Gamma-glutamyl transferase (GGT) activity increased between group of <6 hr and 6 to 12 hr but then decreased between groups of 6 to 12 hr and 12 to 24 hr. The rise was significant between < 6 hr and 6 to 12 hr groups (p value < 0.05) but the decrease between groups of 6 to 12 hr and 12 to 24 hr was not significant. On comparing between < 6 hr and 12 to 24 hr group a rise was seen but found to be statistically insignificant (p value > 0.05). A positive correlation was noted between GGT activity and time since death (r-value +0.290) though statistically not significant.

### Lactate Dehydrogenase (LDH):

Mean value of Lactate Dehydrogenase (LDH) activity increased between groups of <6 hr and 6 to 12 hr and also between groups of 6 to

12 hours and 12 to 24 hr. However, on comparing three groups, between themselves as above none of the rise was statistically significant ( $p$  value  $> 0.05$ ). A positive correlation was noted between LDH activity and time since death ( $r$ -value  $+0.226$ ) although statistically in-significant.

## DISCUSSION

Estimation of enzyme activity in different body fluids like blood, pericardial fluid and CSF to estimate time since death has been done by various workers in the past. In addition, pericardial fluid has also been studied for changes in its constituents with time since death, difference between natural and violent deaths, post-mortem diagnosis of myocardial infarction, cause of death and relation of mechanism of death and nature of pericardial fluid sediment. Some authors have supported the fact that level of pericardial fluid enzymes increases with time elapsed since death whereas some have out rightly refuted the fact. The advantage of pericardial fluid over other body fluids is the large amount available for analysis, the ease of collection and its fluid nature enabling equal distribution of its constituents. The disadvantages are that as it is centrally located in thorax, any pathology or injury to surrounding organs could have an effect on pericardial fluid constituents; furthermore as it contains heart with chambers containing blood hence located relatively close to blood and the microbes that invade blood after death. In the present study the enzymes that were selected were Amylase, Creatine Kinase (CK), Gamma-glutamyl transferase (GGT) and Lactate Dehydrogenase (LDH)- two of cardiac origin (CK, LDH) and two of non-cardiac origin (Amylase and GGT). The hypothesis was as the process of autolysis sets in after death, the intracellular enzymes of cardiac origin will be released into pericardial fluid and their levels would rise with increasing post-mortem interval whereas levels of enzymes of non-cardiac origin will not be raised. Contrary to our hypothesis, in the present study the enzyme activity of all the four enzymes increased with the duration of death though up to different extents. As a rise in all the four enzymes was noted, so the results of this study were contrary to our hypothesis. The possibility of pancreatic (Amylase) and hepatic (GGT) origin of enzymes cannot be ruled out, perhaps the mechanism being diffusion through diaphragm and pericardium into the pericardial fluid. In this study rise in CK was found to be statistically significant. The results of this study showed that of the four studied enzymes, CK and LDH would likely be of use for estimating post-mortem interval for the conditions under which this study was performed.

## CONCLUSION

The regression equations of the present study could not be compared with other studies done in the past as none of the authors had established a regression equation for enzymes in pericardial fluid after death. There is need for further studies keeping more of pre-mortem variables standardized.

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