Original Research Paper

A Study of Pericardial Fluid Enzymes Activities after Death and their Correlation with Post-Mortem Interval

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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 prospective study was undertaken in SMS Hospital, Jaipur on activity of Pericardial Fluid enzymes after death in deceased. A total of 50 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.

Key Words: 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. [1-3] 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.

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Material and Methods:

A total of 50 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 [4-8] 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. [9]

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.

Observations and Results:

Total 50 cases were studied (45 male, 5 female) of age range 8-70 years with maximum cases belonging to 20-30 year age group (36 cases). The manner of injury sustained was wide- ranging from Road traffic accident (39 cases), Fall from height (4 cases), train accident (3 cases), assault (2 cases), hanging (1 case) and fall of heavy weight (1 case).

We compared mean of enzyme activity of all four enzymes (Amylase, CK, GGT and LDH) with time passed since death (Table 1) and statistical significance of enzyme activity changes between three ranges of time since death (<6 hrs, 6-12 hrs and 12-24 hrs) (tables 2 to 5). 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 (table-5).

Creatine Kinase (CK):

Mean value of Creatine Kinase (CK) activity increased between groups of <6 hr and 6 to 12hr 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 [10-12], pericardial fluid [14, 15] and CSF [13] 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 [14, 15], difference between natural and violent deaths [16], post-mortem diagnosis of myocardial infarction [17], cause of death [18] and relation of mechanism of death and nature of pericardial fluid sediment. [19] Some authors have supported the fact that level of pericardial fluid enzymes increases with time elapsed since death [14, 15] whereas some have out rightly refuted the fact. [2]

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 [20-22], 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. (Fig. 1-4)

Though a rise in all the four enzymes was noted but no equation to predict post-mortem interval can be derived as the distribution of enzyme levels at same post-mortem interval was very wide. (Fig. 1-4) The possible reason being that there were many variables in the cases selected for this study i.e.- manner of injury, regions of body injured, mode of death, duration of survival after fatal injury, and periods for which dead body was kept in natural environment and deep freeze prior to autopsy and atmospheric temperature.

The regression equations of the present study could not be compared with other studies done in the past as none of the authors [14, 15] had established a regression equation for enzymes in pericardial fluid after death.

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.

There is need for further studies keeping more of pre-postmortem variables standardized. This study is presented as a pilot study in this relatively less investigated subject and hopefully should pave the way for more elaborate work.

References:

1. **Parikh CK.** Parikh's textbook of medical jurisprudence and toxicology- for classrooms and courtrooms. 6th ed. Delhi, India: Satish Kumar Jain for CBS publishers; 1999
2. **Reddy KSN.** The essentials of forensic medicine and toxicology: 29th ed. Hyderabad, India: K Suguna Devi; 2010.
3. **Vij K.** Textbook of Forensic Medicine and Toxicology: Principles and Practice. 4th ed. New Delhi; Elsevier: 2008.
4. **Ganong WF.** Review of medical physiology. 19th ed. Stamford, Connecticut: Appleton & Lange; 1999.
5. **Guyton AC, Hall JE.** Textbook of Medical physiology, 22nd ed. Singapore: Mc Graw Hill Int. Ed. Harcourt Asia PTE Ltd.; 2005 pg 590-4.
6. **Stewart RH, Rohn DA, Allen SJ, Laine GL.** Basic determinants of epicardial transudation. Am J Physiol 1997, 273: H1408-14
7. **Gibson AT, Segal MB.** A study of the composition of pericardial fluid, with special reference to the probable mechanism of fluid formation: J Physiol 1978 April; 277: 367–377.
8. **Hansen JT, Koeppen BM.** Netter's Atlas of human physiology 1st ed, Icon learning systems LLC, a subsidiary of multimedia, New Jersy: 2002.
9. **Golwalla AF, Golwalla SA.** Golwalla, Medicine for students. 22nd ed. Mumbai, India, 2008. 871-4,390, 285-6, 47-92, 620-42, 92-5,508,673
10. **Arnason A, Bjarnasono.** Postmortem changes in human serum estrase. Acta Pathol Microbiol Scand 1972; 80: 841-6
11. **Garg SDP, Arora A, Dubey BP**. A study of serum enzymal changes after death and its correlation with time since death: J Ind Acad Forensic Med, 2005: 27 (1), ISSN 0971-0973
12. **Lythgoe AS.** Postmortem Activity of Alkaline Phosphatase in Serum from Different Sites of the Cadaver Cardiovascular System .J For Sci Soc 1981; 21 (4): 337- 340.
13. **Dito WR.** Transaminase activity in postmortem cerebrospinal fluid: The American Journal of Clinical Pathology 1964 Oct;42(4):360-3.
14. **Balasooriya BAW, Hill Cast, Williams AR**. The biochemical changes in pericardial fluid after death, an investigation of the relationship between the time since death and the rise of fall in electrolytes and enzyme concentrations and their possible usefulness in determining the time of death. Forensic Sci Int 1984; 26: 93-102
15. **Singh D, Prasad R.** Relationship between the postmortem intervals and the pericardial enzyme activities in subjects of Chandigarh zone of India- a preliminary study. J Indian Acad Forensic Med 2009; 31 (1): 30-36.
16. Arroyo A, Valero J, Marrón T, Vidal C, Hontecillas B, Bernal J. Pericardial fluid postmortem: Comparative study of natural and violent deaths. Am J. Forensic Med Pathol.1998 Sep; 19(3):266-8.
17. **Luna A, Villanueva E, Castellano Ma, Jimenez G.** The determination of CK, LDH and its isoenzymes in pericardial fluid and its application to the post-mortem diagnosis of myocardial infarction: Forensic Sci Int 1982; 19 (1): 85-91.
18. **Luna A, Carmona A, Villanueva E.** The postmortem determination of CK isozymes in the pericardial fluid in various causes of death. Forensic Sci Int 1983; 22: 23-30
19. Luna A, Gomez Zapata M, Vicente V, Osuna E. A cytologic study of the sediments in pericardial fluid as it relates to a diagnosis of the mechanism of death. Z Rechtsmed. 1987; 99(2):129-34.
20. **Chaurasia B D.** Human Anatomy Regional and Applied, Volume one, upper limb and thorax, 3rd ed., CBS Publishers and distributors, Pg.213-215.
21. **Fuster V, Rourke RA, Walsh RA, Wilson PP**. editors,Hurst's The Heart 12th ed, Mc Graw Hill Medical; 2009: 1951-4.
22. **Standring S.** Gray's Anatomy, The anatomical basis of clinical practice. 29th ed. Philadelphia, USA: Elsevier Churchill Livingstone, 2009: 995-6.

Fig. 1: Pericardial Fluid Amylase Enzyme Activity with Post-Mortem Interval

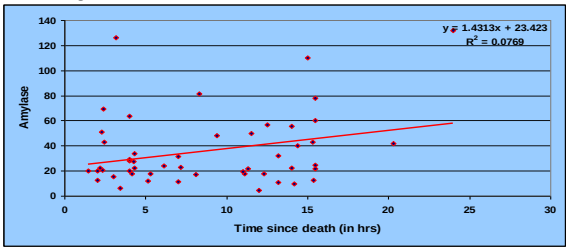

Fig. 2: Pericardial Fluid CK Enzyme Activity with Post-Mortem Interval

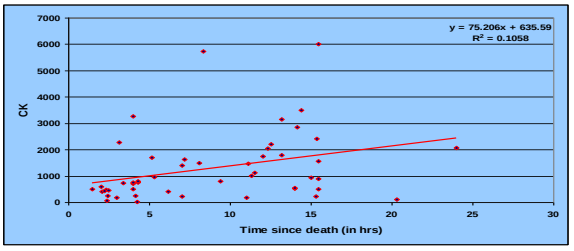

Fig. 3: Pericardial Fluid GGT Enzyme Activity with Post-Mortem Interval

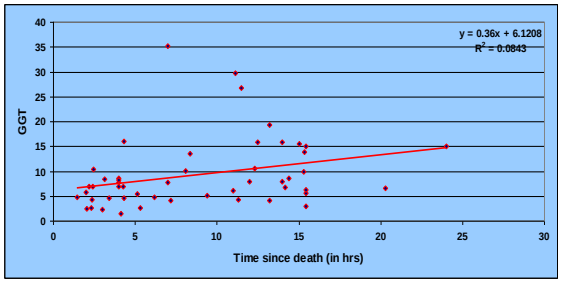

Fig. 4: Pericardial Fluid LDH Enzyme Activity with Post-Mortem Interval

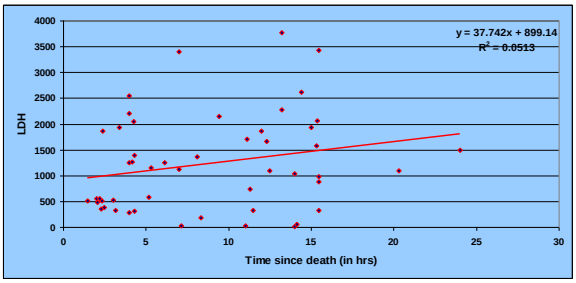

Table 1
Comparison of Mean + SD of enzymes (Amylase, CK, GGT, LDH) with the Post-mortem Interval

Enzyme	Mean + SD U/L		
	Time passed since death (in hrs)		
	< 6 hr (n=21)	6 to <12 hrs (n=11)	12 to24 hrs(n=18)
Amylase	32.43+ 26.43	31.40 + 19.63	43.00 + 34.00
CK	769.34+752.73	1412.43 + 1456.11	1842.05+ 1413.47
GGT	6.02 + 3.29	13.41 + 11.00	10.44 + 4.72
LDH	1002.45+711.83	1117.92 + 987.96	1566.94 + 1005.32

Table 2
Mean + SD of Enzymes (Amylase, CK, GGT, LDH) according to Post-Mortem Interval

Enzyme	Mean + SD U/L		p-value	Significance
	Time passed since death (hrs)			
	< 6 hr (n=21)	6-12hr(n=11)		
Amylase	32.43 + 26.43	31.40 + 19.63	> .05	NS
CK	769.34 + 752.73	1412.43 + 1456.11	> .05	NS
GGT	6.02 + 3.29	13.41 + 11.00	< .05	Significant
LDH	1002.45 + 711.83	1117.92 + 987.96	> .05	NS

Table 3
Mean + SD of Enzymes (Amylase, CK, GGT, LDH) according to Post-Mortem Interval

Enzyme	Mean + SD U/L		p-value	Significance
	Time passed since death (hrs)			
	< 6 hr (n=21)	>12hr (n=18)		
Amylase	32.43 + 26.43	43.00 + 34.00	> .05	NS
CK	769.34 + 752.73	1842.05 + 1413.47	<.01	Significant
GGT	6.02 + 3.29	10.44 + 4.72	> .05	NS
LDH	1002.45 + 711.83	1566.94 + 1005.32	> .05	NS

Table 4
Mean + SD of Amylase, CK, GGT, LDH according to Post-Mortem Interval

Enzyme	Mean + SD U/L		p-value	Significance
	Time passed since death (hrs)			
	6-12hrs (n=11)	>12hrs(n=18)		
Amylase	31.40 + 19.63	43.00 + 34.00	> .05	NS
CK	1412.43 + 1456.11	1842.05 + 1413.47	> .05	NS
GGT	13.41 + 11.00	10.44 + 4.72	> .05	NS
LDH	1117.92 + 987.96	1566.94 + 1005.32	> .05	NS

Table 5
Comparison between Enzyme Activities (Amylase, CK, GGT, LDH) with Post-Mortem Interval and their derived Regression Equations

Correlation	r- Value	p- Value	Significance	Regression Equation
Post-mortem interval v/s Amylase	+0.277	>0.05	NS	Y=1.4313 x + 23.423
Post-mortem interval v/s CK	+ 0.325	<0.05	significant	Y=75.206 x + 635.59
Post-mortem interval v/s GGT	+0.290	>0.05	NS	Y=0.36 x + 6.1208
Post-mortem interval v/s LDH	+0.226	>0.05	NS	Y=37.742 x + 899.14