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Serum and tissue enzymatic changes after death and its correlation with Postmortem interval in goat

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Abstract

Post-mortem interval (PMI) is the time that has elapsed since the animal has died. Estimation of post-mortem interval (PMI) is very important for forensic cases or in veterolegal investigations. The present study was conducted for estimation of post-mortem interval through tissue and serum enzymatic changes after death. Tissue enzymatic changes were studied for ALT and AST in heart as well in liver tissue of goat. Alternation of serum enzymes i.e, AST, ALT, LDH and Alkaline phosphatase were studied after death at different time interval. Tissue enzymatic study of liver & heart revealed gradual increase in AST & ALT values at 6 & 12 hrs. Serum enzymes like AST, ALT, LDH and alkaline phosphatase increased with increase in time after death upto 24 hrs of post death. The changes in these enzymatic values with time passed since death can be used in estimation of post-mortem interval.

Keywords: PMI, goat, ALT, AST, LDH, alkaline phosphatase

Introduction

Post-mortem changes are the changes that occur after death. A proper understanding on post-mortem changes is very important which avoid the risk of misdiagnosis at the time of necropsy. Post-mortem interval (PMI) is the time that has elapsed since the animal has died. Estimation of post-mortem interval (PMI) or time since death is one of the most important objects of post-mortem examination. Estimation of the PMI is critically important in many human death investigations and is similarly relevant in some animal forensic investigations ^[1]. Time passed since death continues to be a major problem for the forensic pathologist and its determination plays an important role in veterolegal cases because forensic experts are very often required to answer questions relating to time of death in the courts of law. There is a continuous need for the development of an accurate method by which the time of death can be determined to within a few minutes ^[2]. Despite many decades of investigation on the topic, accuracy in determination of the time of death has not significantly improved, and no single method can be reliably used to accurately estimate the PMI ^[3, 4]. These enzymatic alterations after death can be helpful in estimation of PMI. Hence the present research work has been undertaken to evaluate serum and tissue enzymatic changes in goats to correlate with time of death.

Materials and methods

The present study was undertaken in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha.

Tissue enzymatic study

Collection of material

Tissue samples like liver and heart were collected from 20 goats immediately after slaughter in slaughter house at an interval of 6 hrs upto 12 hrs. Then 20 g of liver and heart samples washed with sterile normal saline were processed for estimation of ALT & AST enzymes (at 0 hr) and designated as 0 hour sample which was equivalent to antemortem sample. The leftover heart and liver tissue were put in separate sterile beaker each and then kept in normal room temperature. In same process sampling was done at 6 hours and 12 hours for enzymatic study like ALT & AST.

Procedure for ALT & AST estimation

The enzyme estimation (ALT & AST) of heart and liver samples was carried out in three steps i.e. sonication, centrifugation and enzyme assay in air conditioned laboratory.

Sonication: 1 g of heart and liver sample was weighed on electronic balance for estimation of ALT and AST. In glass meat extractor the weighed liver and heart sample were minced with adding PBS of pH 7.2 @ 2 ml/g of sample. During mincing by filling chilled water in piston of meat extractor the temperature of 4-8 °C was maintained. After mincing the mixture was transferred into a cleaned glass beaker and then this beaker was kept in ice pack containing beaker such that 4 °C temperature was maintained during sonication. In ultrasonic processor sonication was done at 100 mA for 10 min.

Centrifugation: The sonicated sample was transferred in to a test tube. In refrigerated centrifuge machine it was centrifuged a 4 °C and 10,000 rpm for 20 mins. Then the supernatant fluid was drawn and transferred in to a small pyrex sample vial (screw capped). Estimation of ALT & AST was done with that supernatant fluid i.e. tissue homogenate.

Enzyme assay: Then with this homogenate of liver and heart sample In spectrophotometer concentration of ALT & AST

was measured in spectrophotometer with kits supplied CPC Biomedicals Pvt. Ltd.

Serum enzymatic studies

Collection of material

Blood samples were collected from 6 dead goats in accidental cases of apparently healthy herd at 0 hr, 3hr, 6hr, 12hr and 24 hr. Postmortem blood sample were collected by piercing jugular vein, femoral vein, Common Carotid artery and on dissection from chambers of Heart with the help of 5ml disposable syringe. Blood samples were kept in slanting manner in the room temperature for 3-4 hours. Then it was centrifuged, serum was separated, labeled and kept in -20° C for further analysis.

Procedure for estimation of LDH, AST, ALT, and ASP

Serum samples were analyzed for estimating LDH, AST, ALT, and ASP in autoanalyzer by using kits of CPC Biomedicals Pvt. Ltd.

Results

Tissue enzymatic study

The mean ± SE values of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentration in the liver and heart samples collected from 20 goats at 0-hour, 6-hours and 12-hours of slaughter are presented in the Table 1.

Table 1: Mean ±SE value of AST and ALT changes in liver and heart after death (P<0.05)

Enzymes	0 hour	6 hours	12 hours
AST (IU/L) (Liver)	67.65 ^c ± 2.77	103.35 ^b ± 3.49	131.20 ^a ± 4.70
ALT(IU/L) (Liver)	269.10 ^c ± 3.61	301.72 ^b ± 4.59	336.25 ^a ± 4.49
AST (IU/L) (Heart)	151.28 ^c ± 3.44	174.99 ^b ± 3.58	209.45 ^a ± 5.47
ALT(IU/L) (Heart)	231.72 ^c ± 2.00	268.05 ^b ± 3.39	308.79 ^a ± 4.45

Aspartate aminotransferase (AST) concentration in liver

The mean values of AST level in the liver at 0-hour, 6-hours and 12 hours of slaughter were 67.65 ± 2.77, 103.35 ± 3.49, 131.20 ± 4.70 (Table 1, Chart 1) indicating significant increase at 6 hrs and 12 hrs (P<0.05).

Alanine aminotransferase (ALT) concentration in liver

The mean values of ALT levels in the liver at 0-hour, 6-hours and 12 hours of slaughter were 269.10 ± 3.61, 301.72^b ± 4.59 and 336.25^a ± 4.49 (Table 1, Chart 1) showing significant increase from 0 hr to 12 hr (P<0.05). The ALT concentration in the liver increased significantly at 6-hours as well as in 12 hours of slaughter (P<0.05) than 0hr with a significant difference between the groups.

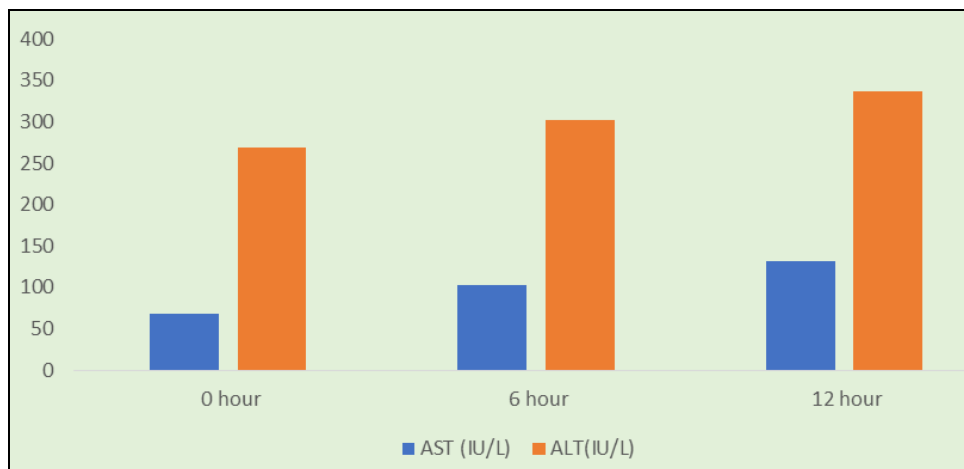


Chart 1: AST and ALT (IU/L) in liver of Goat

In this graph X-axis represents time (in hours) and Y-axis represents concentration of enzymes (AST & ALT) in IU/L.

Aspartate aminotransferase (AST) concentration in heart

The mean values of AST level in the heart were 151.28^c± 3.44, 174.99^b±3.58 and 209.45^a±5.47 IU/L at 0-hour, 6 hour and 12 hours of slaughter (Table 1, Chart 2). The AST concentration in the heart increased significantly at 6-hours and 12 hours of slaughter than 0-hour with a significant difference between

the groups ($P \leq 0.05$).

Alanine aminotransferase (ALT) concentration in heart

The mean values of ALT level in the heart were 231.72^c± 2.00, 268.05^b±3.39 and 308.79^a±4.45 IU/L at 0-hour, 6 hours and 12 hours of slaughter showing significant increase at 6hrs and 12 hours than 0hr with a significant difference between the groups ($P \leq 0.05$) (Table 1, Chart 2).

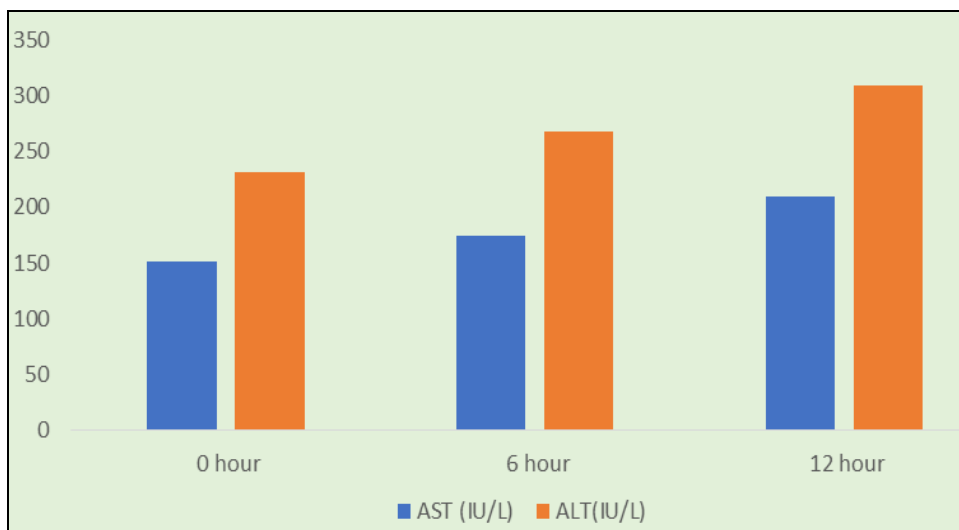


Chart 2: AST and ALT (IU/L) in heart of Goat

In this graph X-axis represents time (in hours) & Y-axis represents concentration of enzymes (AST & ALT) in IU/L.

Serum enzymatic study

The average values of all biochemical parameters i.e. AST,

ALT, ALP and LDH at different time intervals after death are presented in (Table no.2 and Chart 3). Data in different time interval were expressed as mean ± SE, with $P < 0.05$ considered as statistically significant.

Table 2: Mean ±SE value of different Biochemical enzymes of serum in post-mortem blood ($P \leq 0.05$)

Biochemical Parameter	0 hour	3 hours	6hours	12 hours	24 hours
AST (IU/L)	97.33 ^d ± 6.33	117.17 ^c ±9.29	137.00 ^c ±8.09	168.50 ^b ±8.25	222.83 ^a ±12.79
ALT(IU/L)	23.5 ^e ±1.74	35.50 ^d ±2.40	49.83 ^b ±5.19	83.83 ^b ±4.35	133.50 ^a ±4.70
ALP (IU/L)	98.00 ^d ±12.12	116.17 ^d ±14.65	166.67 ^c ±9.15	207.67 ^b ±12.38	261.17 ^a ±6.44
LDH (IU/L)	92.00 ^d ± 9.51	111.83 ^d ±6.19	162.6 ^c ±4.35	192 ^b ±4.43	256.67 ^a ±6.28

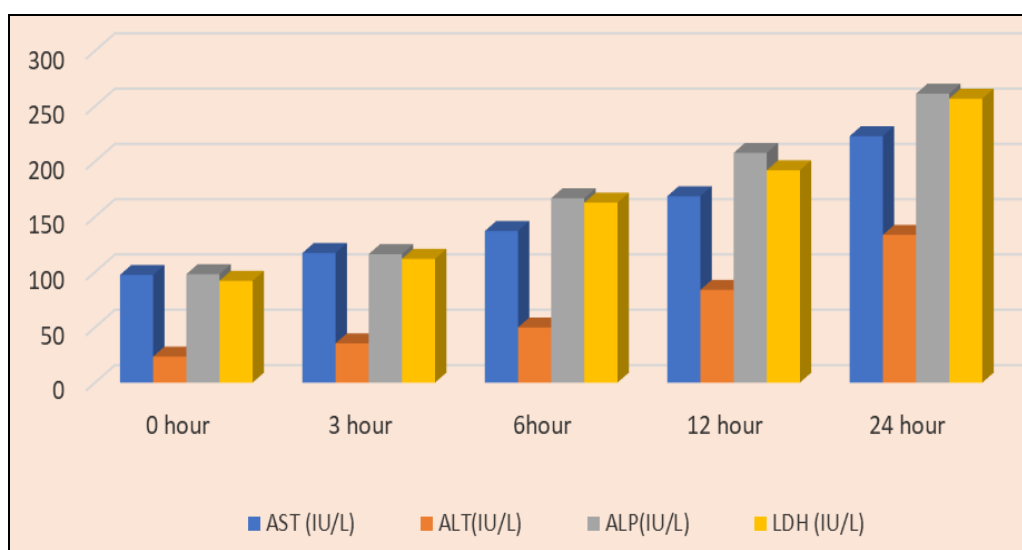


Chart 3: Serum AST, ALT, ALP, LDH (IU/L) comparison with time of death

In this graph X-axis represents time (in hours) and Y-axis represents concentration of enzymes (AST, ALT, ALP & LDH) in IU/L.

Aspartate aminotransferase (AST)

Serum AST (IU/L) values in 0 hours, 3 hours, 6 hours, 12

hours and 24 hours post-mortem were 97.33 ± 6.33 , 117.17 ± 9.29 , 137.00 ± 8.09 , 168.50 ± 8.25 and 222.83 ± 12.79 (Table.2, Chart 4). There was significant increase in serum AST values at 3hrs, 6hrs, 12hrs and 24hrs ($P \leq 0.05$) than 0hr with a significant difference between the groups.

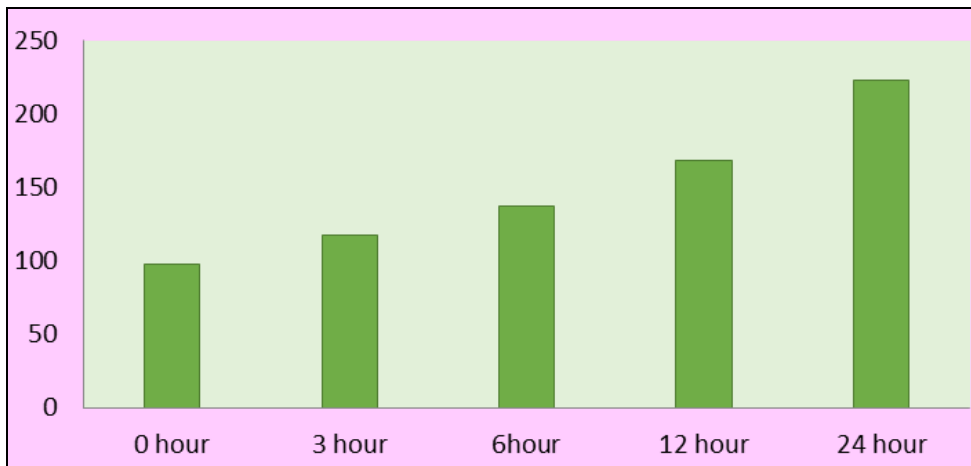


Chart 4: Serum AST(IU/L) comparison with time of death

In this graph X-axis represents time (in hours) & Y-axis represents concentration of AST in IU/L.

Alanine aminotransferase (ALT)

Serum ALT (IU/L) values in 0 hours, 3 hours, 6 hours, 12 hours and 24 hours post-mortem were 23.5 ± 1.74 , 35.50 ± 2.40 ,

49.83 ± 5.19 , 83.83 ± 4.35 and 133.50 ± 4.70 (Table. 2, Chart 5). These values of different time interval showed significant difference and also there was significant increase at 3hrs, 6hrs, 12hrs and 24hr than 0hr ($P \leq 0.05$).

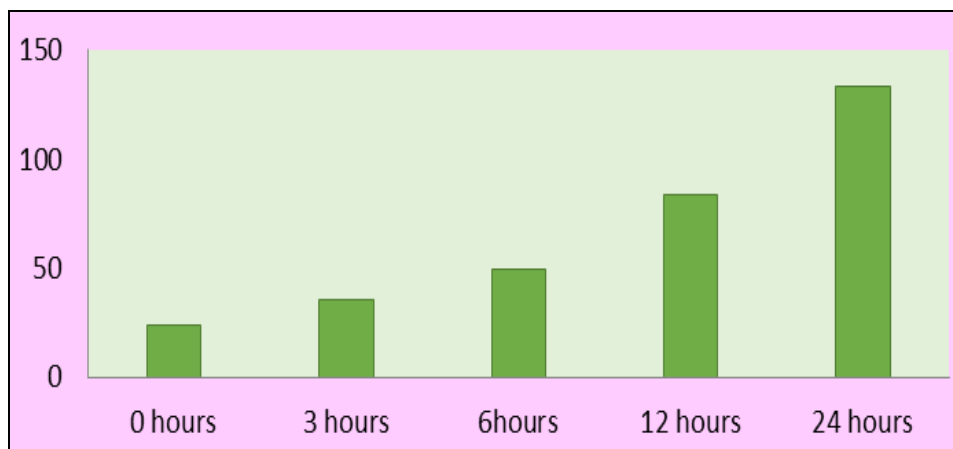


Chart 5: Serum ALT (IU/L) Comparison with time since death

In this graph X-axis represents time (in hours) & Y-axis represents concentration of ALT in IU/L.

Alkaline phosphatase

Serum alkaline phosphatase (IU/L) values in 0 hours, 3hours, 6 hours, 12 hours and 24 hours post-mortem were $98.00 \pm$

12.12 , 116.17 ± 14.65 , 166.67 ± 9.15 , 207.67 ± 12.38 and 261.17 ± 6.44 (Table. 2, Chart 6). There was significant increase in serum Alkaline phosphatase values at 3hrs, 6hrs, 12hrs and 24hrs than 0hr with a significant difference between the groups of 6 hours, 12 hours and 24 hours time ($P \leq 0.05$).

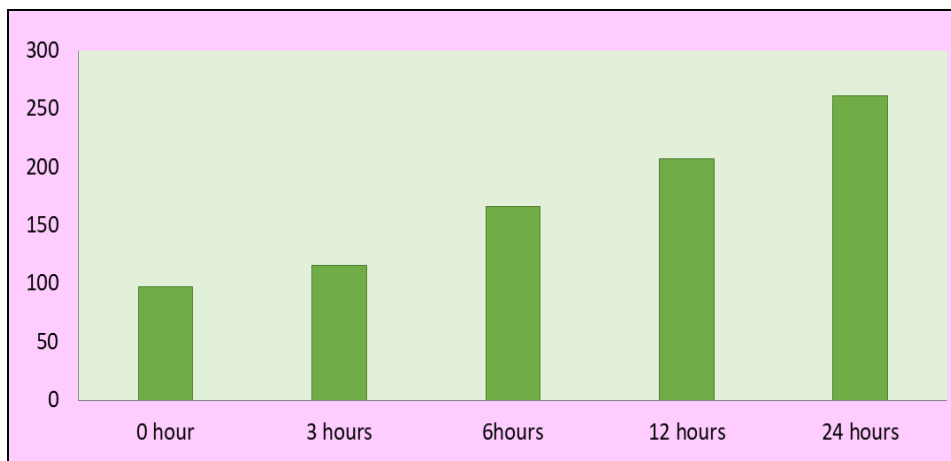


Chart 6: Serum Alkaline phosphatase (IU/L) comparison with time since death

In this graph X-axis represents time in hours & Y-axis represents concentration of serum alkaline phosphatase in IU/L.

hours and 24 hours post-mortem were 92.00 ± 9.51, 111.83 ± 6.19, 162.67 ± 4.35, 192 ± 4.43 and 256.67 ± 6.28 (Table. 2, Chart 7). There was significant increase in serum LDH values at 3hrs, 6hrs, 12hrs and 24hrs than 0hr ($P \leq 0.05$).

Lactate dehydrogenase (LDH)

Serum LDH (IU/L) values in 0 hours, 3 hours, 6 hours, 12

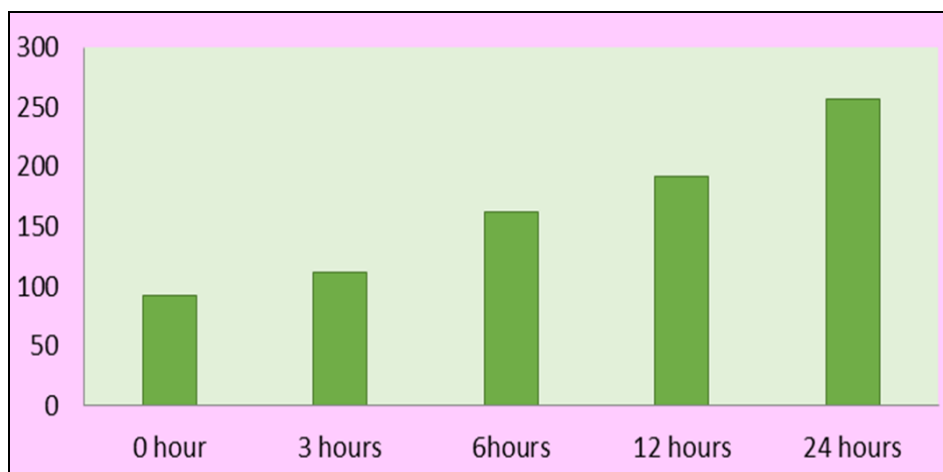


Chart 6: Serum LDH (IU/L) comparison with time since death

In this graph X-axis represents time in hours & Y-axis represents concentration of LDH in IU/L.

Discussion

For accurate determination of post-mortem interval, tissue enzymatic parameters like alanine aminotransferase (AST) and alanine aminotransferase (ALT) concentration in the liver and heart samples collected from 20 goats at 0-hour, 6-hours and 12 hours of slaughter were evaluated. From the result it was found that there was significant increase in ALT and AST in liver as well as in heart tissues in 6 hours and 12 hours. There was rising and declining trends of AST and ALT in liver and heart tissue followed by the same or reverse pattern in the post-mortem interval between 4-38 hours [5]. Normally these enzymes present in cytosol, mitochondria etc., but after death there is cell disintegration, autolysis or purification of the tissue so these enzymes are released hence on estimation their concentration rises. So this may be the reason of sharp increase of these tissue enzymes at 6 hrs and 12 hrs of death. Serum samples were analysed for different serum enzymes i.e., ALT, AST, LDH, Alkaline phosphatase from 6 post-mortem cases. Serum samples were taken in different time

interval (0 hour, 3 hours, 6 hours, 12 hours and 24 hours) after death. The result showed that there was gradual significant increase in serum AST, ALT, Alkaline phosphatase and LDH values at 3hrs, 6hrs, 12hrs and 24hrs than 0hr. According to the previous research it was reported that serum Aspartate amino transferase and Alanine aminotransferase are of clinical importance. Normally the serum transaminases levels are low but after extensive tissue damage these enzymes are liberated into serum. So AST and ALT in serum rises after death due to post-mortem damage of tissues [6]. There was rising trend of serum aminotransferase levels up to second to third day of death [7]. The concentration rose from 8 kA units just after death to 40 kA units after 30 hours and then rose steeply up to 40 hours when it recorded a peak of 70 kA units and afterwards fell downwards [8]. Changes in Lactate Dehydrogenase levels in serum after death and found that there was almost linear increase in concentration of this enzyme up to well over 60 hours and peak may not be attained until fourth day and because of linear rise which may be useful in calculating time since death [9]. All these enzymatic values increase with time passed from death. As after death there is autolysis, purification of different organs

so the enzymes releases to blood and their values rises than normal [7, 9].

It can be concluded from the present study that these changes in enzymatic values in serum as well as in tissues after death can be correlated with time passed since death which helps in estimation of PMI. Further more research is needed in this topic for more accuracy.

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