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# Screening biochemical markers from muscles as PMI indicator

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**ABSTRACT**

Post-mortem interval (PMI), or time since death, is a matter of crucial importance in any death investigation. Recent developments in biochemical technologies have started to identify biomarkers in different biological fluids such as blood, urine and tissues such as muscle, liver, brain for PMI estimation. The present study focusing on the use of muscle in PMI estimation suggest that the muscle was examined biochemically for enzyme like alkaline phosphatase, alanine aminotransferase, aspartate amino transferase, lactic acid, catalase etc. *Labeo calbasu* fishes were sacrificed for estimating activity of bimolecular markers to determine post-mortem changes in their concentration at the intervals of 0, 3, 4.5, 6 and 24 hrs. Enzyme activity levels so obtained were charted and statistically studied and graphical records were obtained against post-mortem interval. Use of these biochemical markers could be promising tools to determine Post-mortem interval. In this study we observed insignificant correlation between the *post-mortem* concentration of biochemical parameters such as alkaline phosphatase, alanine aminotransferase, aspartate amino transferase, lactic acid, catalase due to effects of certain factors such as age, condition of fish, sex etc.

***Key words :*** *Post-mortem interval, Biomolecular markers, Enzyme activity, Alkaline phosphatase, Alanine aminotransferase, Aspartate amino transferase, Lactic acid, catalase*

## Introduction

The estimation of the post-mortem interval (PMI) by biochemical means is based on the analysis of chemical substances which are released after death and accumulate in the body. If any substance can be measured accurately and correlated with the time since death, it might provide a method of determining the PMI. Within a few minutes of death, autolysis, or cell disruption begins with the release of water, of which the body is mainly composed, and enzymes, which begin the degradation of proteins, lipids, and carbohydrates (Hayman and Oxenham, 2016). The total amount of protein in the muscles has been considered as a marker for time of death but was found to be variable (Donaldson and Lamont, 2014). Skeletal muscle enzymes or proteins are markers of the functional status of muscle tissue, and vary widely in both pathological and physiological conditions (Brancaccio *et al.,* 2010).

The biochemical markers that have shown change over time after death in the muscle will be discussed in this study.

### Alkaline phosphatase (ALP)

Alkaline phosphatases are plasma membrane-bound glycoproteins. Alkaline phosphatase (ALP) is an enzyme present in various tissues throughout the body. It is primarily found in cell membranes where active transport processes occur, such as the endothelium of arterioles and endomysial capillaries. ALP is a generic name for phosphormonoesterases that

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hydrolyse orthophosphate at an alkaline PH. These enzymes are widely distributed, usually activated by magnesium, manganese, zinc, and cobalt ions, and inhibited by cysteine, cyanides, and arsenates (Webb, 2021).

**Alanine aminotransferase (ALT)**

Alanine aminotransferase (ALT) is the most widely used clinical biomarker of hepatic health. As its name implies, ALT is involved in the transamination of alanine, and it is present in the liver at much higher concentrations than in other organs. ALT is a transaminase enzyme that was formerly known as serum glutamate pyruvate transaminase (SGPT). It catalyses the transfer of an amino group from alanine to alpha-ketoglutarate in the alanine cycle to form pyruvate and glutamate. It is found in serum and organ tissues, especially liver, although significant concentrations are also found in kidney, skeletal muscle, and myocardium ("Chapter 3 Clinical Biochemistry and Hematology | Elsevier Enhanced Reader," n.d.).

**Aspartate Aminotransferase**

Aspartate aminotransferase (AST) is a transaminase enzyme that catalyses the conversion of aspartate and alpha-ketoglutarate to oxaloacetate and glutamate. AST enzyme was formerly known as serum glutamate oxalate transaminase (SGOT) and is present in all tissues except bone, with highest levels in liver and skeletal muscle. Concentration of AST is elevated after bruising, trauma, necrosis, infection, or neoplasia of liver or muscle (Eugster et al., 1966). It catalyse the reaction that converts aspartic acid to glutamate. AST is found in the heart and skeletal muscle, as well as liver, kidney, erythrocytes, and brain tissue. AST is used as an indicator of liver damage due to necropsy or carcinogenesis as it is released from the tissues into the extracellular space and this will also occur post mortem. The amount of AST enzyme released from any tissue depends on the severity of cellular damage, so that a crude PMI estimate was possible (Donaldson and Lamont, 2014).

**Lactic acid**

Lactic acid is produced in the muscles of several species of fish examined and of lobsters under conditions in which it is known to be produced in other animals already studied (Ritchie, 1927). The quantity present is less than in the skeletal muscles of the land-living vertebrates (Ritchie, 1927). The amount of lactic acid developed in muscular tissue during *rigor mortis* or during artificially produced *rigor* is fairly constant for any one type of muscle and has a characteristic value for any one species (Ritchie, 1927). In general, under conditions of extreme activity, when the energy demand exceeds the capacity of aerobic energy production, anaerobic metabolism is activated in order to produce additional ATP. Due to a high free energy loss, the reaction has a fast reaction rate and is nearly independent of substrate and product levels. It is reported by Ritchie, (1927) that the store of lactic acid precursor in fish muscle is small and is exhausted during the normal *post mortem* changes.

**Catalase**

Catalase is an antioxidant enzyme present in all aerobic organisms. It is known to catalyse $H_2O_2$ into water and oxygen in an energy-efficient manner in the cells exposed to environmental stress. Catalase is located in all major sites of $H_2O_2$ production (Sharma and Ahmad, 2014). It protects tissues against damage by hydrogen peroxide (Kumari *et al*., 2014). Aerobic living cells produce the highly reactive oxidants superoxide anion, hydrogen peroxide and singlet oxygen. These metabolites are toxic for the cells by causing oxidations of unsaturated lipids, amino acids and nucleic acids Several enzymes protect against toxic oxidation. Hydrogen peroxide is decomposed by catalase, and glutathione peroxidase acts on both hydrogen peroxide and organic peroxides (Aksnes and Njaa, 1981).

**Muscle tissue as potential for the source of biomolecular markers**

It has been well established by Reznick *et al.* (1989) that considerable changes in the structure and function of aging skeletal muscles take place, involving both biochemical and morphological parameters. According to histological appearance, innervation, and morphological relationships, the body muscles of fishes must be classified with the striated muscle of land-living vertebrates, but their functions more closely resemble those of rhythmically contracting heart or smooth muscle. Thus, present study focuses on the ALP, AST, ALT, lactic acid and catalase derived from the muscle tissue of *Labeo calbasu.*

Kalibaush (*Labeo calbasu*) is a teleost fish species distributed in Bangladesh, India, Pakistan, Myanmar, Thailand, and also South China. Once the

species was abundant in all natural water bodies. During the early 1980s, *Labeo calbasu* was of great commercial importance like 3 other Indian Major Carps- *Labeo rohita*, *Gibelion catla*, and *Cirrhinus mrigal* (Hossain et al., 2010). *Labeo calbasu* is a major species of carp family and have a high commercial importance. It is a bottom feeder and can easily be reared in any freshwater ponds, lakes and deep pools of tributaries (Basak and Hadiuzzaman, 2019). However, in the recent years, the natural breeding of *L. calbasu* has become uncertain due to continuous habitat degradation caused by environmental modification and human interventions (overfishing, dam construction, pollution etc.) affecting feeding migration and spawning which decreased its population size (Mostafa *et al*., 2009).

This work focuses on use of biochemical markers in the muscles that change in the post-mortem interval. The purpose of the present study to examine the activity of ALP, AST, ALT, lactic acid and catalase in muscle tissue of *Labeo calbasu* so as to reconfirm the role of these markers in determining PMI.

## Materials and Methodology

### Material

Fifteen *Labeo calbasu* fishes were obtained from the breeder and acclimatized in the laboratory for 8 days. They were randomly divided in to three groups, each having five individuals. All of them were sacrificed at once by destructing the brain by pithing. Independent fresh muscle samples were collected from the sacrificed fishes. All chemicals were reagent grade or equivalent for catalase and lactic acid analysis. The biochemical parameters were quantified using a Biuret Method Erba Alkaline Phosphatase Kit for ALP, ARKRAY MBK kit for GPT (ALT) and GOT(AST). Lactic acid was estimated using colorimetric method of Miller and Muntz (Hullin and Noble, 1953). Catalase activity was determined by finding the rate constant using iodometric method.

### Sample preparation and purification

#### For ALP, ALT, AST and Catalase

Three fishes were taken at a time at the time interval of 0, 1 hr, 3 hrs, 4.5 hrs and 24 hrs from death. The fishes were deskinned and muscle were pulled out. Muscle samples were homogenized using mortar and pestle in 0.1M phosphate buffer followed by centrifugation to 10000g for 30 minutes at 4° C and supernatant collected was used for further colorimetric analysis of ALP using IFCC method, AST and ALT by NADH assay and catalase by iodometric method.

#### For lactic acid

Samples were homogenized in 0.6M chilled perchloric acid using mortar and pestle followed by centrifugation to 6000g for 10 minutes at 4°C. Supernatant collected for further analysis using a colorimetric method.

## Results and Discussion

The present study was to investigate the biochemical changes in muscles within 24 hours after death (as shown in Table 1). All three sets showed different enzyme activities at zero hour indicating that their initial levels vary from fish to fish.

Alkaline phosphatase activity was same for all the sets at zero hour and it did not show any change till 6 hrs. However, the activity vanished after 24 hours as showed in Fig. 1.

Aspartate Transaminase showed fall in enzyme activity till 4.5 hours of death, which was considerably elevated at 6$^{th}$ hour and again decreased sharply till 24 hours. Though, a similar pattern of activity is seen, there was large difference in the enzyme activity in each set. The activity was significantly different in each fish as showed in Fig. 2.

**Table 1**. Enzyme activity w.r.t. post-mortem interval of Labeo calbasu

| PMI (in | ALP(IU/L) | | | AST(IU/L) | | | ALT(IU/L) | | | Catalase activity Units/Sec | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| hours) | Set 1 | Set 2 | Set 3 | Set 1 | Set 2 | Set 3 | Set 1 | Set 2 | Set 3 | Set 1 | Set 2 | Set 3 |
| 0 | 27.64 | 27.64 | 27.64 | 115.4 | 75 | 210 | 22.8 | 6 | 159.6 | 0.008 | 0.0035 | 0.026 |
| 3 | 27.64 | 27.64 | 27.64 | 28.85 | 106.28 | 1410 | 208.22 | 12 | 188.2 | 0.012 | 0.048 | 0.0269 |
| 4.5 | 27.64 | 27.64 | 27.64 | 11.54 | 56.25 | 0 | 387.6 | 48 | 228.8 | 0.0259 | 0.0384 | 0.0429 |
| 6 | 165.84 | 27.64 | 27.64 | 138.48 | 918.75 | 1650 | 484.4 | 60 | 91.2 | 0.0777 | 0.025 | 0.0625 |
| 24 | 0 | 0 | 0 | 86.55 | 675 | 210 | 136.8 | 10 | 68.4 | 0.005 | 0.001 | 0.0582 |

**Table 2.** Activity of Lactic acid W.R.T. Post-Mortem Interval of *Labeo calbasu*

| PMI (in hours) | Lactic acid (µg/ml) | | |
|---|---|---|---|
| | Set 1 | Set 2 | Set 3 |
| 0 | 19 | 55 | 10 |
| 3 | 25 | 15 | 6 |
| 4.5 | 70 | 117 | 20 |
| 6 | 56 | 62 | 15 |
| 24 | 48 | 19 | 10 |

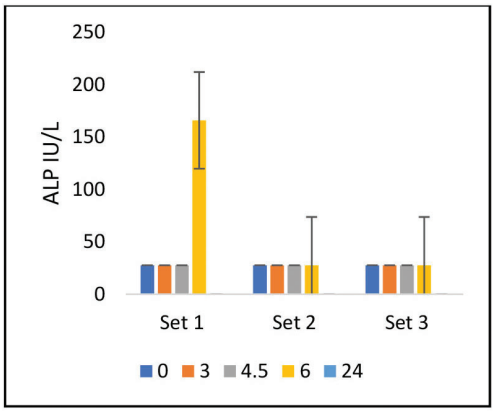


**Fig. 1.** ALP IU/L w.r.t PMI

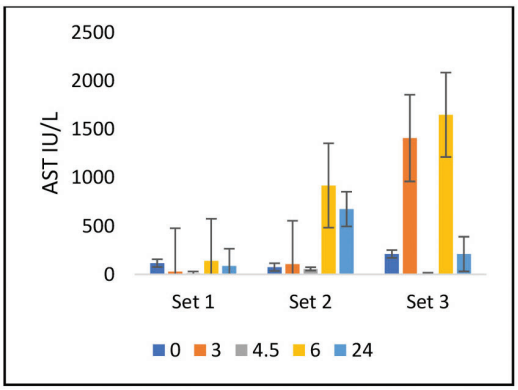


**Fig. 2.** AST IU/L w.r.t PMI

Alanine transaminase also showed rise in its activity till 4 Hours but the significant fall was noticed at 24 hrs (Figure 3). Similarly, the lactic acid also found to be increasing till 6 Hours followed by considerable declining at 24 hrs as showed in figure 4.

The catalase activity also showed the same trend of increasing till 6 hrs and then fall on 24$^{th}$ hour as seen in Figure 5.

The three sets were not significant to each other at 95% confidence level indicating that each individual fish has different enzyme regime which depends on its own physiological condition.

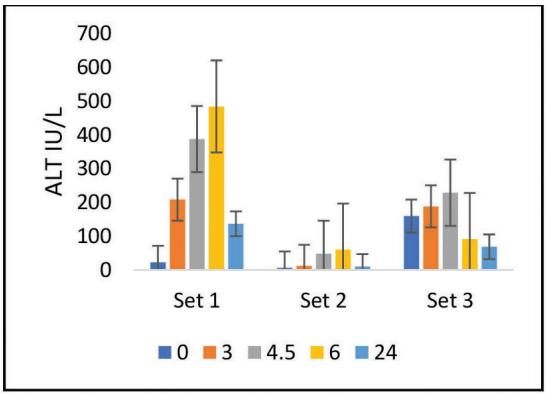


**Fig. 3.** ALT IU/L w.r.t PMI

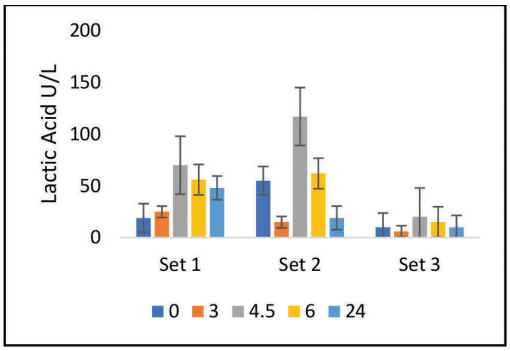


**Fig. 4.** Lactic acid (µg/ml) w.r.t PMI

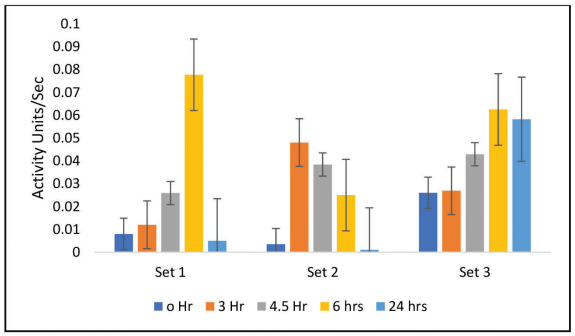


**Fig. 5.** Catalase activity Units/Sec w.r.t PMI

The use of liver function markers from blood and post-mortem reliability of liver function was also studies by (Fumeaux *et al.*, 2018), which indicates elevation in the levels of AST and ALT in post-mortem samples irrespective of post-mortem samples. The present study also indicates similar trend. However, there is fall in these enzymes on 24$^{th}$ hour. The lack of data from 6$^{th}$ hour till 24 hrs makes it difficult to understand exactly from when the enzyme degradation started. A study performed on heart and liver tissue of goat also showed increase in AST, ALT and ALP levels after death upto

12 hrs (Das *et al.*, n.d.). The research by (Costa *et al.*, 2015) indicates significant fluctuations in the levels of AST, ALT and ALP in the in post mortem human venous blood samples. The lactic acid was found to be linearly elevating in the samples of post mortem individuals who were suffering from diabetes upto 8-10 days according to (Keltanen *et al.*, 2015) who suggest that it is because of autolysis and bacterial metabolism. Linear increase is also found in the calf plasma who was still born (Jawor *et al.*, 2019). However, in the present study, there is elevation only upto 4 hrs followed by its fall which is not in accordance to above discussion. The varying timing of these changes makes PMI determination problematic as the nature, or presence, of each stage of decomposition is strongly influenced by endogenous and environmental factors such as temperature, humidity, age and sex (Madea and Musshoff, 2007).

Variation in the catalase activity is varied with the species as reported by Anders Aksne (Aksnes and Njaa, 1981). As per the work (Paltian *et al.*, 2019), catalase activity in Swiss mice skeletal muscle showed no difference.

## Conclusion

Although for practical purposes, there has been no real breakthrough in estimating the time of death by these biomoleculare markers; however, no such studies found on *Labeo calbasu* fish. No significant trend of variation was seen in the selected enzymes which will help in determining exact time after death. Every individual fish has different initial concentrations of the selected enzymes which does not allow to interpret the PMI and also indicate that every individual fish has its own enzyme pathophysiological condition. Most of the enzymes increased upto 6 hrs and then at 24$^{th}$ hr the rapid fall was noticed. The missing data between 6 to 24 hrs is not allowing the authors to comment of exact time of start of decline in the activity.

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### Conflict of Interest

Authors are not directly or indirectly related to the work submitted for publication.

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