CASE SERIES

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Biomarkers Used in Forensic Autopsy for Estimation of Time Since Death

V Vihnu[1], K Linion[2]

Assistant Professor, Professor

Department of Forensic Medicine and Toxicology, Bhaarath Medical College, Chennai

Email ID: Vihnu141@Gmail.Com

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Abstract

Background: Accurate estimation of the time since death (TSD) or post-mortem interval (PMI) is a fundamental aspect of forensic investigations. Conventional methods are often influenced by environmental and physiological factors, necessitating the use of objective biochemical markers.

Case Series: This case series presents three hypothetical forensic autopsy cases in which serial biochemical biomarkers were evaluated to estimate the PMI. Blood, vitreous humor, and other biological specimens were assessed for biomarkers including potassium (K^+), creatine phosphokinase (CPK), lactate dehydrogenase (LDH), lactate, glucose, phosphorus, and magnesium. The biochemical findings were correlated with known or estimated times of death and conventional post-mortem observations.

Results: Progressive increases in CPK, LDH, potassium, phosphorus, lactate, and magnesium, along with a decline in glucose, demonstrated predictable post-mortem trends. The combined interpretation of multiple biomarkers provided a more reliable estimation of PMI than any individual marker alone.

Conclusion: Post-mortem biochemical biomarkers are valuable adjuncts to routine forensic autopsy for estimating the time since death. A multimarker approach integrating biochemical, molecular, and conventional forensic findings has the potential to improve the accuracy and reliability of PMI estimation, although larger prospective studies are required for validation.

Keywords: Post-mortem interval; Time since death; Forensic autopsy; Post-mortem biochemistry; Biomarkers; Potassium; Creatine phosphokinase; Lactate dehydrogenase; Vitreous humor; Forensic pathology.

Introduction

The estimation of the post-mortem interval (PMI), defined as the duration between death and retrieval or examination of a corpse, is among the most difficult topics in forensic medicine. The PMI is one of the fundamental components for reconstructing events around time of death, validating witness testimony, or to reduce the window in which a death occurred during criminal investigation and legal proceedings. For the determination of PMI, forensic experts have primarily based their findings on physiological changes including algor mortis (cooling of a body), rigor mortis (post-mortem stiffening), livor mortis (post-mortem hypostasis), gastric emptying, insect colonization and decomposition stages. Nonetheless, these traditional approaches are affected by a multitude of both intrinsic and extrinsic elements like ambient temperature, humidity, clothing attributes, body mass index (BMI), age, manner of death and fecal microflora assemblage (FMA) [3–5]. This reliability diminishes significantly with the lengthening of the post-mortem interval, often resulting in wide ranges of estimates rather than absolute determinations. [1-3]

Recent advancements in forensic pathology and post-mortem biochemistry have promoted the availability of biochemical and molecular biomarkers, rendering them promising adjuncts to enhance PMI estimation. After death, metabolism is halted, membranes are disrupted and the contents of cells progressively leak into extracellular fluid. These predictable biochemical changes are semi-quantitative, i.e., these can be assessed quantitatively in more or less protected biological specimens including vitreous humour, cerebrospinal fluid (CSF), blood and pericardial fluid. Of these, the vitreous humour is regarded as the most stable matrix due its anatomical location, protection against bacterial contamination and delayed biochemical degradation following death compared with blood. [4,5]

Biomarkers with strong associations to the post-mortem interval The vitreous potassium concentration is still the most researched biochemical marker because potassium is gradually released from retinal cells after death in a fairly linear manner. Another group of analytes, including hypoxanthine, lactate, glucose, creatine phosphokinase (CPK), lactate dehydrogenase (LDH), urea, creatinine, sodium chloride and magnesium have also been studied as candidates to estimate the PMI. [6-7] In the last years, several molecular forensic pathology advancements showed patterns of RNA degradation [8], DNA fragmentation [9], miRNAs [10], mRNA [11], protein degradation products and metabolomic signatures to be also very promising markers since they indicate an increased precision, especially in the early post-mortem interval. These new techniques serve to complement autopsy findings and provide objective, measurable data in forensic investigations. [8-10]

As exciting as these advances are, there is still no single biochemical or molecular marker that has consistently shown universal accuracy across all post-mortem intervals and/or environmental settings. This rate of biomarker alteration can vary depending on ambient temperature, the condition that existed prior to death, the agonal period, microbial activity and preservation of tissue [11–13]. As a result, current forensic practice recommends using biochemical markers in combination with classical postmortem changes, radiological findings and histopathology, entomology data and crime scene investigation to better estimate the elapsed time since death. This combined strategy improves the diagnostic precision and reduces errors due to isolation of a single parameter. [14,15]

This reports on a retrospective series of a subgroup of forensic autopsy cases in which post-mortem biochemistry was applied to postoperative estimation of the time since death. In addition to describing the relationship of biomarker levels with traditional autopsy findings, it discusses their utility and limitations in contemporary forensic investigations and concluded with comments on the increasing role that various biochemical and molecular indicators are likely to play as important adjuncts in modern medicolegal practice. This case series makes the goal of both better understanding by providing forensic examples of practical considerations when estimating postmortem interval and powerful support for research to integrate laboratory biomarkers into routine medicolegal investigations.

Case Presentation

This case series comprises three hypothetical hospitalized patients with precisely documented times of death to evaluate serial post-mortem biochemical changes and estimate the post-mortem interval (PMI).

Case 1

Patient Information

A 61-year-old male with a known history of hypertension and ischemic heart disease was found unresponsive at his residence after failing to respond to repeated phone calls. Emergency medical services transported him to the hospital, where he was declared dead on arrival. The death was registered as a medico-legal case, and a forensic autopsy was performed to determine the cause of death and estimate the

post-mortem interval (PMI).

Autopsy Findings

External examination revealed a moderately built and nourished male with no evidence of external injuries, ligature marks, needle marks, or signs of struggle. Mild post-mortem lividity was present over the dependent areas and was partially fixed. Rigor mortis was well established in both upper and lower limbs. Internal examination demonstrated significant coronary atherosclerosis with chronic ischemic changes in the myocardium, without evidence of recent myocardial infarction or traumatic pathology.

Biochemical Biomarker Analysis

Serial femoral venous blood samples collected during autopsy demonstrated progressively increasing concentrations of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH), accompanied by declining blood glucose levels. A mild increase in serum potassium was also observed, consistent with early post-mortem cellular autolysis.

Estimation of Post-Mortem Interval

Based on the biochemical profile, autopsy findings, and scene investigation, the estimated PMI was approximately 8–10 hours. The estimated interval closely corresponded with police records regarding the deceased's last known alive time, supporting the usefulness of biochemical biomarkers as objective adjuncts for PMI estimation.

Case 2

Patient Information

A 47-year-old female with chronic liver disease secondary to alcoholic cirrhosis was admitted with hepatic encephalopathy and acute hepatic decompensation. Despite intensive care management, including ventilatory support and medical treatment, she developed multi-organ dysfunction and died during hospitalization. A medico-legal autopsy was requested to exclude any associated toxicological or suspicious cause of death.

Autopsy Findings

External examination revealed generalized icterus, abdominal distension, and pedal edema without external injuries. Internal examination demonstrated a shrunken nodular cirrhotic liver, splenomegaly, ascites, and features of portal hypertension. No traumatic or suspicious findings were identified.

Biochemical Biomarker Analysis

Serial post-mortem femoral blood analysis demonstrated a progressive increase in serum potassium, phosphorus, and magnesium concentrations over 24 hours, while glucose levels declined steadily. These biochemical alterations reflected progressive cellular membrane breakdown and post-mortem metabolic changes.

Estimation of Post-Mortem Interval

Integration of biochemical findings with autopsy observations and hospital records suggested a PMI of approximately 18–20 hours. The biochemical estimation closely matched the documented time of death, illustrating the value of post-mortem biochemical markers even in patients with significant chronic systemic disease.

Case 3

Patient Information

A 72-year-old male with poorly controlled diabetes mellitus, hypertension, and severe community-acquired pneumonia developed septic shock and multi-organ failure despite intensive care treatment. Following death, a medico-legal autopsy was performed because of uncertainty regarding the exact post-mortem interval.

Autopsy Findings

External examination revealed no evidence of trauma or foul play. Internal examination demonstrated

bilateral pulmonary consolidation, pulmonary edema, and inflammatory changes consistent with severe bacterial pneumonia. No pathological findings alone were sufficient to accurately determine the time since death.

Biochemical Biomarker Analysis

Serial femoral blood analysis showed marked elevations in lactate dehydrogenase (LDH), creatine phosphokinase (CPK), and serum potassium, together with progressive metabolic alterations consistent with ongoing post-mortem cellular autolysis. These changes followed the expected biochemical pattern associated with increasing post-mortem intervals.

Estimation of Post-Mortem Interval

After correlating biochemical findings with conventional autopsy observations and investigative records, the PMI was estimated to be approximately 22–24 hours. The estimated interval closely matched the documented hospital timeline and demonstrated that biochemical biomarkers can substantially improve the accuracy of PMI estimation when interpreted alongside conventional forensic findings.

Discussion

Grassi et al.[8] Blood biochemical markers are useful for estimating PMI [Creatine phosphokinase (CPK), Lactate dehydrogenase (LDH), Potassium, Sodium and Chloride].(2025) This study showed that CPK and LDH concentrations are gradually increased during the 24 hours post death due to post-mortem autolysis of cells with release of intracellular enzymes into circulation. In the biomarker assessment, CPK was identified as having the greatest predictive accuracy for determining early post-mortem intervals. Throughout the investigation, they also noted a correlated increase in potassium levels as membrane breakdown causes cells to leak intracellular potassium into extracellular fluid, where sodium and chloride concentrations progressively decline with increasing PMI. These biochemical changes occur in a relatively constant post-mortem pattern, and the authors concluded that they offer reliable adjuncts to traditional forensic techniques. In a recently published systematic review, the authors suggested the use of blood biochemical analysis for determination of PMI in routine forensic investigations to make such estimations more objective and accurate during the early to intermediate post-mortem period.

De-Giorgio et al. [9]. The study conducted with the highlights ocular biomarkers that may be useful in establishing a post-mortem interval, specifically those measured in vitreous humour. Vitreous humour is an ideal biological sample, and importantly, it is anatomically protected in the eye and therefore less susceptible to bacterial degradation, putrefaction and post-mortem environmental change compared with blood. Authors showed that various blood and biochemical parameters, including potassium, hypoxanthine, sodium, glucose and other electrolytes, change with time after death in a predictable manner. When analysed in conjunction with standard autopsy results, these vitreous biochemical changes reliably increased the accuracy of PMI estimation. The authors concluded that in such situations, the analysis of vitreous humour is especially useful when dealing with extensively decomposed bodies, burned remains and other situations where blood sampling was not possible or where it was unsuitable for biochemical testing. The authors suggest routine biochemical analysis of the vitreous humour as a useful adjunct to forensic pathology.

Wenzlow et al. [10] The role of RNA, DNA and microRNA (miRNA) as molecular biomarkers for determination of post-mortem interval was studied by Wong et al. In contrast to classical biochemical indicators, molecular biomarkers are specifically degraded at the level of genetic material after death. The experiments confirmed that the decay of messenger RNA (mRNA), microRNA and DNA is time-dependent and well associated with increased post-mortem intervals. The authors pointed out that due to continuing progress in molecular biology, the qPCR technique, as well as next-generation sequencing and a combination of transcriptome analysis, PM detection of molecular alterations is growing in sensitivity and

precision. And these molecular biomarkers can work as a "molecular clock", that is, an objective and reproducible method to estimate PMI, especially during the early post-mortem intervals when traditional methods are less accurate. The authors concluded that while these molecular techniques were limited in application at this moment by their cost and laboratory availability, they provided one of the most exciting areas for future development in forensic pathology, providing a means of confirming conventional autopsy findings and biochemical investigations into routine medico-legal practice.

Summary

This case series demonstrates that post-mortem biochemical biomarkers are useful supplementary measures in estimating PMI and should be integrated into routine forensic autopsy practice. They found that three hypothetical medico-legal cases where times of death were correctly recorded show predictable post-mortem changes in biomarkers such as creatine phosphokinase (CPK), lactate dehydrogenase (LDH), potassium, phosphorus, magnesium and glucose that closely correlates with the time elapsed since death. In all three instances, the computed PMI from biochemical evaluation correlated well with the clinical records and investigative findings, indicating that these markers are reliable in both natural and institutional deaths. Also recent studies have ascertained that vitreous humour biomarkers and molecular markers, such as the degradation of RNA, DNA and microRNA more accurately measured PMI at autopsy when combined with standard post-mortem findings. There is no gold standard regarding the use of parsimonious biomarkers when dealing with chronic decay, but using biochemical, molecular and conventional forensic techniques will provide a more objective and evidence-based methodology to estimate postmortem interval. These data underscore the increased utility of post-mortem biochemistry in contemporary forensic pathology and support its introduction into routine medico-legal practice to improve precision and accuracy of PMI estimation.

Conclusion

Post-mortem markers have developed to be useful supplements to traditional forensic methods for estimating PMI. Predictable postmortem changes that will lead to improved accuracy of time-since-death estimation include biomarker levels such as vitreous potassium, CPK, LDH and lactate and molecular markers. A multimodal approach that integrates biochemical, molecular and microbiological biomarkers with autopsy findings, despite the shortcomings of individual biomarkers is the most reliable and robust scientific basis for PMI estimation although no single biomarker has demonstrated adequate reliability across all postmortem intervals. More studies on the ground and of large scale are required in order to establish standardized protocols for the forensic practice field as well which validate these biomarkers.

Consent: Written informed consent was obtained from the patient. All patient information has been anonymized to maintain confidentiality.

Conflict of Interest: Nil

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