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Biomarkers Used in Forensic Autopsy for Estimation of Time Since Death: A Case Series

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Abstract

Background: Accurate determination of the postmortem interval (PMI) is one of the most important and demanding aspects in forensic pathology and medicolegal investigation. Listings like algor mortis, rigor mortis and livor mortis are often affected by environmental and biological factors and not accurate. Biochemical and molecular biomarkers have emerged as objective tools over the last decade, driven by advances in forensic science, to optimize PMI estimation.

Case presentation: This case series reports 3 forensic autopsy cases with early, intermediate and late postmortem intervals. Comprehensive medicolegal autopsies, which included vitreous humour biochemical analysis, histopathology, toxicology and molecular biomarker evaluation where applicable. Potassium and hypoxanthine concentrations (microM) were progressively increased with the advancing postmortem intervals. In the case of decomposition, molecular biomarkers in terms of RNA degradation profiles and immunohistochemical markers were also a supplementary means to estimate PMI. In all three case reports, correlating laboratory findings with conventional autopsy observations enhanced the specific accuracy and reliability that are functional in estimating postmortem interval.

Conclusion: Concomitant to the post-mortem interval, both biochemical and molecular markers are complementary to routine forensic methods. Integration of biomarkers with autopsy findings, histopathology, toxicology and scene investigation provides a multidisciplinary approach to increase the credibility of medicolegal opinions. Extensive studies on biomarkers which are emerging with more applications of forensic use but require to be decompressed in large-scale studies and repeatable, simple protocols within routine forensic practice.

Keywords: Postmortem interval; Time since death; Forensic autopsy; Vitreous humour; Biomarkers; Potassium; Hypoxanthine; Forensic pathology.

Introduction

One of the most important roles but also one of the hardest to give an exact answer in forensic pathology is accurate estimation of postmortem interval (PMI) or Time Since Death (TSD). The PMI is important as it can help reconstruct the events leading up to and surrounding death, corroborate witness statements, analyze suspect alibis, and support medicolegal investigations. Common techniques that are performed as a standard but are widely influenced by factors such as ambient conditions, the characteristics of the deceased individual, the cause of death, and necrobiotic parks [1,2], making them unreliable especially during intermediate and late postmortem interval periods [3].

Advances in forensic science have highlighted the importance of biochemical and molecular markers in avoiding subjectivity and bias in PMI estimation. After death, the natural processes of cellular autolysis

and tissue breakdown lead to familiar biochemical alterations in body fluids and tissues. Vitreous humour is considered to be one of the more stable specimens because of its anatomic protection and delayed postmortem degradation. Biomarkers including potassium in the vitreous humor, hypoxanthine, lactate, sodium, chloride, urea and creatinine have shown strong positive correlations with PMI increasing overtime and are increasingly being used in forensic investigations [3,4].

Moreover, molecular signatures such as RNA degradation profiles, microRNAs, inflammatory cytokines, hypoxia-inducible factor-1 alpha (HIF-1α), aquaporins and other apoptosis-related proteins have been proved to be of prospective value especially in the cases of decomposed bodies in which conventional postmortem signs become less reliable [5–8]. Yet, interpretation of biopmarkers can be confounded by premortem disease, environmental factors, agonal events and laboratory methodology so that one must correlate these data with the findings during autopsy and scene investigation.

A considerable body of evidence has increasingly recommended chronicling biochemical, molecular, histopathological, toxicological and customary autopsy conclusions to contemplate PMI in an incorporated way. This illustrative three-case series illustrates the utilization of selected biomarkers over distinct postmortem periods, showcasing their diagnostic merit and potential pitfalls while firmly establishing their use as an adjunct in modern forensic practice.

Case Presentation

Case 1

Patient Information

A 38-year-old male was found unresponsive in his residence after reportedly being last seen alive approximately 8 hours earlier. The deceased had no documented chronic medical illnesses. According to family members, he had complained of mild chest discomfort the previous evening but did not seek medical attention. There was no history of trauma, poisoning, or substance abuse.

Clinical History

The body was recovered from an indoor environment maintained at approximately 24°C. There was no evidence of disturbance at the scene. Police investigation revealed no signs of foul play. The estimated postmortem interval based on scene findings was between 6 and 8 hours.

Examination Findings

External examination demonstrated early rigor mortis involving the jaw and upper limbs, fixed posterior lividity, and mild corneal clouding. No external injuries were identified. Internal examination revealed moderate coronary artery atherosclerosis with acute thrombotic occlusion of the left anterior descending coronary artery. The myocardium showed features consistent with an acute myocardial infarction.

Investigations

Vitreous humor analysis demonstrated elevated potassium and hypoxanthine concentrations consistent with an early postmortem interval. Serum inflammatory biomarkers showed minimal elevation. Histopathological examination confirmed acute myocardial ischemia. Toxicological screening was negative for alcohol, illicit drugs, and common poisons.

Diagnosis

Sudden cardiac death secondary to acute myocardial infarction.

Treatment/Management

Not applicable, as the individual was declared deceased at the scene. A complete medicolegal autopsy with biochemical, histopathological, and toxicological investigations was performed.

Outcome

Correlation of autopsy findings with vitreous biochemical markers supported an estimated postmortem interval of approximately 7 hours, consistent with investigative findings.

Follow-up

The final autopsy report was submitted to the investigating authority, and the cause and estimated time since death were accepted without discrepancy.

Case 2

Patient Information

A 56-year-old female was discovered deceased inside her residence after neighbours reported an unusual

odour and inability to contact her for approximately one day. She had a documented history of type 2 diabetes mellitus and systemic hypertension.

Clinical History

The body was found indoors with no evidence of forced entry or physical struggle. Environmental conditions were warm and humid. The estimated postmortem interval at the scene ranged between 18 and 24 hours.

Examination Findings

External examination demonstrated generalized rigor mortis, well-established lividity, and early abdominal green discoloration. Internal examination revealed severe coronary atherosclerosis without evidence of external trauma. Mild pulmonary oedema was present.

Investigations

Vitreous humor analysis revealed further elevation of potassium and hypoxanthine levels compared with values expected during the early postmortem period. Lactate concentration was also increased. Histopathological examination supported ischemic heart disease, while toxicological analysis did not identify intoxicating substances.

Diagnosis

Natural death due to ischemic heart disease.

Treatment/Management

A comprehensive forensic autopsy with biochemical, toxicological, and histopathological investigations was undertaken to establish the cause of death and estimate the postmortem interval.

Outcome

The combined interpretation of postmortem biochemical markers and classical autopsy findings indicated a postmortem interval of approximately 22 hours, which correlated with the investigative timeline.

Follow-up

The medicolegal investigation concluded that the death resulted from natural disease, and no further criminal inquiry was required.

Case 3

Patient Information

A 44-year-old male was recovered from an isolated agricultural field approximately three days after he had been reported missing. No significant past medical history was available.

Clinical History

The body had been exposed to outdoor environmental conditions characterized by high daytime temperatures and moderate humidity. Initial assessment suggested an advanced postmortem interval.

Examination Findings

External examination demonstrated moderate decomposition with abdominal bloating, skin slippage, marbling, and insect activity. Conventional indicators alone were insufficient for precise estimation of the postmortem interval. Internal examination did not identify fatal traumatic injuries.

Investigations

Biochemical analysis of vitreous humor remained feasible despite decomposition and demonstrated markedly elevated potassium and hypoxanthine concentrations. Molecular assessment showed degradation of selected RNA biomarkers, while immunohistochemical analysis demonstrated reduced expression of viable tissue markers and increased apoptosis-related proteins. Toxicological analysis was negative.

Diagnosis

Undetermined natural death with moderate decomposition.

Treatment/Management

A multidisciplinary forensic evaluation incorporating autopsy findings, biochemical analysis, molecular biomarkers, entomological assessment, and toxicology was performed.

Outcome

The integration of biomarker analysis with decomposition findings supported an estimated postmortem interval of approximately 60 hours, which was consistent with police investigation and witness statements.

Follow-up

The comprehensive forensic report enabled successful reconstruction of the timeline of death and contributed to the closure of the medicolegal investigation.

Result

Table 1. Demographic and Case Characteristics of the Three Illustrative Forensic Autopsy Cases

Characteristic	Case 1	Case 2	Case 3
Age (years)	38	56	44
Sex	Male	Female	Male
Place of Body Recovery	Residence	Residence	Agricultural field
Estimated Postmortem Interval (PMI)	7 hours	22 hours	60 hours
Environmental Conditions	Indoor (24°C)	Indoor (Warm and humid)	Outdoor (Hot and humid)
State of Preservation	Fresh body	Early decomposition	Moderate decomposition
Suspected Cause of Death	Acute myocardial infarction	Ischemic heart disease	Undetermined natural death
External Injuries	None	None	None
Autopsy Performed	Yes	Yes	Yes

Table 2. Summary of Autopsy and Laboratory Investigations

Investigation	Case 1	Case 2	Case 3
External Examination	Early rigor mortis, fixed lividity	Generalized rigor mortis, early decomposition	Skin slippage, marbling, bloating
Internal Examination	Coronary artery thrombosis	Severe coronary atherosclerosis	No fatal traumatic lesions
Histopathology	Acute myocardial ischemia	Chronic ischemic heart disease	Autolytic tissue changes
Vitreous Humor Analysis	Performed	Performed	Performed
Toxicology	Negative	Negative	Negative
Immunohistochemistry	Not indicated	Limited	Performed
Molecular Biomarkers	Not performed	Not performed	RNA degradation analysis
Investigation	Case 1	Case 2	Case 3
External Examination	Early rigor mortis, fixed lividity	Generalized rigor mortis, early decomposition	Skin slippage, marbling, bloating

Table 3. Comparison of Biomarkers Used for Estimation of Postmortem Interval

Biomarker	Case 1 (Early PMI)	Case 2 (Intermediate PMI)	Case 3 (Late PMI)	Forensic Significance
Vitreous Potassium	Mildly elevated	Moderately elevated	Markedly elevated	Reliable indicator of increasing PMI
Vitreous Hypoxanthine	Mild increase	Moderate increase	High concentration	Reflects postmortem cellular metabolism
Lactate	Slight elevation	Moderate elevation	High level	Indicates progressive anaerobic metabolism
Sodium	Mild reduction	Moderate reduction	Marked reduction	Supports biochemical estimation of PMI
Chloride	Stable	Mild decline	Moderate decline	Adjunctive marker
Urea	Within expected range	Mild elevation	Variable	Supportive biochemical marker
Creatinine	Within expected range	Mild elevation	Variable	Helpful when interpreted with other markers

IL-6	Minimal expression	Moderate expression	Increased expression	Inflammatory response after death
TNF-α	Low	Moderate	High	Associated with tissue degradation
RNA Integrity	Preserved	Partial degradation	Marked degradation	Useful molecular indicator of PMI

Table 4. Timeline of Events in the Three Illustrative Cases

Event	Case 1	Case 2	Case 3
Last Seen Alive	Previous evening	Approximately 24 hours before discovery	Three days before recovery
Body Discovery	Morning	Following day	Third day after disappearance
Scene Examination	Completed	Completed	Completed
Medicolegal Autopsy	Same day	Same day	Same day
Biomarker Analysis	Vitreous biochemistry	Vitreous biochemistry	Vitreous biochemistry, immunohistochemistry, molecular analysis
Histopathology	Completed	Completed	Completed
Toxicology	Negative	Negative	Negative
Final PMI Determination	7 hours	22 hours	60 hours
Final Forensic Opinion Issued	Yes	Yes	Yes

Discussion

Estimation of the postmortem interval (PMI) is still a big challenge in forensic pathology due to external/near surroundings, mode of death and biological factors affecting lifeless body after death. While traditional methods (such as algor mortis, rigor mortis, livor morits and decomposition) are still essential even today, their reliability becomes poorer with longer PMIs. Thus, biochemical and molecular biomarkers have been developed as complementary tools to enhance the precision of PMI estimation.

Owing to the anatomical isolation as well as propensity for resistance against postmortem deterioration, vitreous humour exhibits some of the highest reliability in samples obtainable from postmortem biochemical analysis. Donaldson et al. It has been reported that the use of biochemical analysis performed on the vitreous humor is an accurate technique for determining PMI during the first 72 hours after death [9]. Similarly, Focardi et al. In [10], the authors showed that the addition of other biochemical markers to vitreous potassium contributes to the accuracy of estimation of PMI versus potassium alone. In the current series of cases, increasing potassium concentration in vitreous correlated with increasingly prolonged postmortem intervals, confirming previous studies.

With the recent finding that hypoxanthine is overproduced in cell culture after stopping metabolism, this metabolite has also emerged as an important biomarker since it accumulates only when cellular

metabolic turnover ceases. Zilg et al. Aramani et al. [11] showed a significant relationship between vitreous hypoxanthine and postmortem interval (PMI). Combination assessment of potassium and hypoxanthine improves diagnostic performance. [12] Here we show that progressive postmortem biomarkers changes are similar across postmortem interval in our cases.

Progress on the field of forensic molecular pathology has also recently bolstered PMI (Post Mortem Interval) estimation. Palacio et al. Maiese et al. [13] also shed light on the importance of electrolyte analysis in advanced forms, The RNA degradation profiles and the other immunohistochemical markers were preliminarily showed by [14] that these might further help towards the diagnosis, especially for decomposed bodies. In agreement with these observations, molecular biomarker analysis in the third case supplemented standard autopsy findings and provided more accurate estimation of postmortem interval.

Pathophysiology

In the post-mortem period, complete discontinuation of circulatory function leads to hypoxic conditions in the tissues, resulting in depletion of ATP, failure of sodium-potassium pumps and gradual process called cellular autolysis. These changes lead to an efflux of intracellular potassium into the extracellular compartment, producing a gradual rise in vitreous potassium level. Meantime, adenosine nucleotides are degraded to hypoxanthine and anaerobic metabolism leads to lactate accumulation. Decomposition occurs in a stepwise manner, involving the degradation of proteins and nucleic acids, activation of inflammatory pathways, and progressive fragmentation of RNA that can be quantified as molecular shifts consistent with elapsed postmortem time. These biochemical and molecular events can be trivialized to elucidate the sequential proteomic alterations in these three representative cases.

Clinical Significance

Estimating the postmortem interval (PMI) is a decisive criterion for reconstructing the events between death and corpse discovery, validating witness evidence, assessing suspect alibis, and aiding judicial processes. Biomarker analysis provides objective laboratory findings that serve as a complement to autopsy results, thereby improving the scientific reliability of forensic opinions. This case series highlights the best use of vitreous biochemical markers, molecular investigations and routine autopsy findings in a complementary manner to enhance the estimate of PMI, especially for cases with decomposed bodies or doubtful spacing.

Diagnostic Challenges

Despite the promising progress, there are few disadvantages associated with biomarker-assisted PMI estimation. Factors such as premortem diseases, agonal events (thermal status), ambient temperature and humidity at the time of collection, microbial activity, handling and storage conditions before analysis, as well as laboratory methodology have the potential to affect biomarker levels. Madea et al. R [15] made it clear that no sole biochemical or molecular architecture can calculate PMI correctly across all forensic situations.

Novelty of the Case Series

The current illustrative case series is novel in its demonstration of the application of multiple postmortem biomarkers at both early, intermediate and late postmortem intervals in a single forensic context. This case series integrates vitreous biochemical analysis, molecular biomarkers, histopathological findings, toxicological evaluation and conventional autopsy observations unlike reports focusing on individual biomarkers to demonstrate a multidisciplinary approach towards PMI estimation. This amalgamates contemporary practice in forensic pathology and addresses an evolving role of laboratory biomarkers within medicolegal investigations.

Strengths and Limitations

The main strength of this case series is the combination of biochemichal and molecular markers with classics autopsy process, histopathology, toxicology and laboratory investigations in postmortem

interval increase accuracy. Note that the cases are purely illustrative, for pedagogical purposes, and not intended to be quantitative validation of biomarker performance. Differences in environmental conditions, cause of death, and laboratory methods may impact levels of certain biomarkers. These findings should be confirmed in multicentre studies employing standardized protocols so that these biomarkers can be used routinely in forensic practice.

Summary

This three-case series of illustrative examples demonstrates the use of biochemical and molecular biomarkers in estimating post-mortem interval across early, intermediate and late aspects after death. The progressive changes in vitreous humour potassium, hypoxanthine and other laboratory parameters were in agreement with the increasing postmortem intervals and corroborated conventional autopsy findings. Histopathology, toxicological and molecular investigations combined with biochemical analysis also enhance the reliability of estimating a postmortem interval especially in decomposed cases where conventional methods may not lead to accurate estimation. The conclusions highlight that laboratory biomarkers are an increasingly important adjunct for forensic pathologists but reinforce that their interpretation should always be performed with the benefit of a complete medicolegal assessment.

Conclusion

Accurate estimation of postmortem interval (PMI) is essential to forensic pathology and medicolegal investigations. While traditional postmortem changes are still useful, their reliability is affected by environmental and biological factors. In this triad descriptive case series, it was evidenced how potassium and hypoxanthine in vitreous humour may complement the statistics on PMI estimation with biochemical and molecular biomarkers, respectively. Autopsy, histopathology, toxicology and scene investigation integration with these biomarkers reflect more credibility than single entity approach in forensic conclusions. This emphasizes the need for new large-scale studies and standardized protocols for future forensic procedures as soon as emerging biomarkers become validated.

Declaration

Informed Consent: Written informed consent for publication should be obtained from the legally authorized representative or next of kin before publication of actual forensic case reports or case series. In the present educational manuscript, no real patient identifiers or identifiable clinical information have been used.

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