DOI: https://doi.org/10.53350/pjmhs2023178108

**ORIGINAL ARTICLE**

# Comparative Analysis of Postmortem Biochemical Markers in Vitreous Humor and Cerebrospinal Fluid for Estimating Time of Death

FARHAT SULTANA[1], KHALID MAHMOOD[2], RIASAT ALI[3], FARIHA TARIQ[4], ZUBIA IQBAL[5], AATIQA ABBAS[6]
[1]*Associate Professor Forensic Medicine Department Allam Iqbal Medical College Lahore, Pakistan*
[2]*Assistant professor Forensic Medicine Department Services Institute of Medical Sciences Lahore, Pakistan*
[3,4]*Assistant professor Forensic Medicine Department King Edward Medical University Lahore, Pakistan*
[5]*Assistant Professor Forensic Medicine Department, Ameer Ud Din Medical College / Post Graduate Medical Institute / Lahore General Hospital, Lahore, Pakistan*
[6]*Demonstrator Forensic Medicine Department King Edward Medical University Lahore, Pakistan*
*Correspondence to: Farhat Sultana, Email: farhatzafar42@gmail.com*

## ABSTRACT

**Background:** Forensic investigations in Pakistan that have climatic diversity require accurate estimation of postmortem interval (PMI). Environmental variability makes traditional physical methods unreliable, and as a result biochemical methods using postmortem fluids are used.

**Objective:** This study aimed to compare the efficacy of vitreous humor and cerebrospinal fluid (CSF) biochemical markers as complementary tools in the estimation of PMI in a forensic setting.

**Methods:** n=60 medico-legal autopsy cases were studied prospectively in the Forensic Medicine Departments of Jinnah hospital and Mayo hospital, Lahore, Pakistan. Minimal decomposition and lack of confounding ocular or CNS pathologies were ensured by inclusion and exclusion criteria. CSF and vitreous humor samples were obtained at autopsy within 12 hours postmortem. Vitreous humor potassium, glucose, and lactate levels were measured with ion-selective electrodes, and CSF levels of neuron-specific enolase (NSE) and S100B were determined by ELISA. Ambient temperature and humidity were used as covariates in statistical analyses, including Pearson correlation and multiple linear regression.

**Results:** Vitreous potassium had a strong positive correlation with PMI ($r = 0.85$, $p < 0.001$), explaining 72% of the variance in PMI. The level of S100B was not statistically significant while CSF NSE moderately correlated with an $R^2$ value of 0.45 ($r = 0.68$, $p = 0.002$). These results demonstrate that vitreous potassium is a superior predictor of PMI than CSF markers, which provide complementary information on central nervous system degradation.

**Conclusions:** The results support the use of vitreous humor as the primary fluid for biochemical PMI estimation with CSF markers being useful adjuncts, especially in cases of CNS pathology. Forensic time of death determinations in Pakistan may be improved with a dual fluid approach adjusted for local environmental conditions.

**Keywords:** Postmortem Interval, Vitreous Humor, Cerebrospinal Fluid, Biochemical Markers, Forensic Science, Pakistan

## INTRODUCTION

Forensic investigations of regions with diverse climatic conditions such as in Pakistan rely heavily on the accurate estimation of the postmortem interval (PMI), which is a cornerstone of forensic investigations[1]. Finally, conventional techniques relying on the physical indicators of rigor mortis, livor mortis, and algor mortis are heavily dependent upon environmental factors, individual physiology, and local weather extremes, resulting in inconsistent results. This demonstrates the need for more objective and reliable methodologies in forensic practice[2].

Recent advances in forensic biochemistry have focused analysis of biochemical markers present in postmortem biological fluids. Of these, vitreous humor and cerebrospinal fluid (CSF) have proved to be promising matrices for PMI estimation because they are relatively isolated from systemic changes that occur shortly following death[3]. The vitreous humor is a reliable medium where one can study the progressive diffusion of intracellular components such as potassium which has a predictable increase in concentration after death. On the other hand, CSF is a good source of information about CNS degradation and metabolic changes, which may indicate rapid biochemical changes that cannot be observed in other fluids[4].

The integration of biochemical methods in the context of the Pakistani forensic services is very important where the forensic services are adapting to work under the resource and environmental constraints and the integration of biochemical methods could increase the accuracy of PMI estimation[5]. Recent regional studies have reported encouraging trends regarding the use of vitreous potassium dynamics and CSF neuronal markers to support time of death assessment. However, there is not much comprehensive research comparing the two fluids locally[6].

The objective of this study was to critically and comparatively evaluate the utility and reliability of such biochemical markers in vitreous humor and CSF in Pakistan[7]. This study aimed to examines temporal profiles of key electrolytes and metabolites in both fluids to develop a more accurate model for PMI determination that incorporates the idiosyncratic environmental and infrastructural factors in the region. This study is expected to ultimately contribute to the refinement of forensic methodologies in Pakistan as it will help the quality of legal and investigative outcomes and the field of forensic science to grow within the country[8].

Received on 08-07-2023
Accepted on 27-08-2023

## MATERIALS AND METHODS

**Study Design and Setting:** This was a prospective comparative study that was evaluated over a period of 12 months (June 2022 till June 2023) in the Forensic Medicine Departments of Jinnah hospital and Mayo hospital, Lahore, Pakistan. The study was designed to evaluate and compare postmortem biochemical profiles of vitreous humor and cerebrospinal fluid (CSF) for the improvement of the estimation of postmortem interval (PMI).

**Ethical Considerations:** The study received approval from the Institutional Ethics Committees of both participating institutions. It was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and national regulations governing research on human tissues. Informed written consent was obtained from the legal next of kin after providing detailed information about the study's objectives, procedures, and data confidentiality. To maintain anonymity, all personal identifiers were removed, and each case was assigned a unique code.

**Case Selection:** According to strict inclusion and exclusion criteria, a total of n=60 consecutive medico-legal autopsy cases were included. The cases had to have a reliable time of death based on scene investigation and circumstantial evidence, minimal decomposition to preserve biochemical integrity, and the ability to obtain both vitreous humor and CSF without significant contamination. Cases were excluded if they had extensive ocular trauma, preexisting ocular pathology, advanced decomposition

which might affect marker stability, or known/suspected central nervous system pathology that might confound CSF marker levels.

**Sample Collection:** Immediately after autopsy, vitreous humor was aspirated from each eye using a sterile 21 gauge needle introduced laterally in order to minimise contamination. One to two milliliters per eye was obtained per eye, the samples were pooled into prelabeled sterile vials to minimize variability. To obtain CSF, a suboccipital puncture of the cisterna magna was made using a sterile spinal needle with 2–3 mL collected in prelabeled sterile containers. Both fluids were sampled within 12 hours postmortem to reduce the effects of decomposition.

**Sample Handling and Storage:** The all collected samples were cooled in refrigerated environment at 4 °C and transported to the forensic biochemistry laboratory within two hours. Samples were aliquoted prior to arrival, to avoid repeated freeze thaw cycles and stored at –20°C until analysis. The biochemical markers were kept stable by only one freeze-thaw cycle for each sample.

**Biochemical Analysis:** The biochemical analysis consisted of protein markers, electrolytes, and metabolites quantification. Vitreous humor and CSF potassium levels were determined by using an ion-selective electrode (ISE) analyzer with daily calibration with a certified standard solution to keep intra and inter-assay coefficients of variations below 5%. The concentrations of glucose and lactate were quantified by HPLC with UV detection employing standard curves prepared with certified reference standards and duplicate analyses to ensure reproducibility. Neuron-specific enolase (NSE) and S100 calcium-binding protein B (S100B) levels were determined in CSF samples using commercially available ELISA kits validated for forensic use and each assay was performed in triplicate and read at 450 nm wavelength in a calibrated microplate reader.

**Quality Control and Data Analysis:** Blank samples, duplicate analyses, and standard reference materials were incorporated into every assay run to maintain quality control. The SPSS software (version 25) was used in the recording and statistical analysis of data. All variables were computed for descriptive statistics and regression models were developed for the concentration of biochemical markers and the estimated PMI. In the analysis, ambient temperature and humidity recorded at the time of sample collection were included as covariates. Statistically significant was considered as a p-value of less than 0.05.

## RESULTS

The postmortem biochemical profiles of vitreous and cerebrospinal fluid (CSF) were compared in 60 medico legal autopsy cases. In the following sections, the demographic characteristics, environmental conditions, biochemical markers levels and the statistical analysis performed in order to assess the correlation between these markers and the postmortem interval (PMI) are described.

**Demographic and Environmental Data:** The mean age of the study population was 35 ± 10 years 66.7% (n=40) of cases were male and 33.3% (n=20) were female. The ambient temperature at the time of autopsy was 28 ± 2°C and relative humidity was 60 ± 5%. Subsequent analyses included these variables as covariates.

Table 1: Demographic and Environmental Characteristics (n=60)

| Parameter | Value/Description |
|---|---|
| Mean Age (years) | 35 ± 10 |
| Sex | Male: 40 (66.7%), Female: 20 (33.3%) |
| Ambient Temperature | 28 ± 2 °C |
| Relative Humidity | 60 ± 5 % |

The demographic profile and environmental conditions of the study cases are presented in explanation, Table 1. The male cases predominate the data and the environmental conditions are consistent which are important in adjusting the regression models used in PMI estimation.

**Biochemical Marker Levels:** Both vitreous humor and CSF were measured for the levels of various biochemical markers. Table 2 presents the mean values and standard deviations of the key markers. Vitreous potassium, a well-known marker for PMI, had a mean of 12.5 ± 2.5 mmol/L, vitreous glucose, and lactate at 40 ± 10 mg/dL (3.3 ± 1.1 mmol/L), respectively. Potassium levels in CSF were lower (9.8 ± 1.5 mmol/L) than in vitreous humor. CSF analysis included neuronal markers such as neuron-specific enolase (NSE) with a mean level of 28.5 ± 6.2 ng/mL, and S100 calcium-binding protein B (S100B) with a mean level of 0.75 ± 0.18 µg/L.

Table 2: Biochemical Marker Levels in Vitreous Humor and CSF

| Biochemical Marker | Vitreous Humor | CSF |
|---|---|---|
| Potassium (mmol/L) | 12.5 ± 2.5 | 9.8 ± 1.5 |
| Glucose (mg/dL) | 40 ± 10 | — |
| Lactate (mmol/L) | 5.8 ± 1.2 | — |
| Neuron-Specific Enolase (ng/mL) | — | 28.5 ± 6.2 |
| S100B (µg/L) | — | 0.75 ± 0.18 |

In the two fluids, the mean biochemical concentrations are summarized in Table 2. However, biochemical profiles between vitreous humor and CSF support the notion of different biochemical profiles that could enable a more accurate PMI estimation.

**Statistical Analysis and Correlation with PMI:** A Pearson correlation analysis was done to assess the relationship between the biochemical markers and the estimated PMI. A strong positive correlation was also observed between vitreous levels of potassium and PMI ($r = 0.85$, $p < 0.001$). Likewise, NSE levels in CSF had a moderate correlation with PMI ($r = 0.68$, $p = 0.002$) while S100B was not statistically significant ($r = 0.42$, $p = 0.06$).

Moreover, PMI was predicted with multiple linear regression analyses. The regression coefficient of the regression model using vitreous potassium as a predictor was 0.95 ($p < 0.001$) and the $R^2$ value was 0.72 (which implies that 72% of the variability in PMI could be explained by vitreous potassium levels). The CSF NSE levels were used in a separate regression model, and the regression coefficient was 0.32 ($p = 0.003$), and the $R^2$ value was 0.45.

Table 3: Regression Analysis for Predicting Postmortem Interval (PMI)

| Predictor | Regression Coefficient | Standard Error | p-value | $R^2$ |
|---|---|---|---|---|
| Vitreous Potassium | 0.95 | 0.12 | <0.001 | 0.72 |
| CSF Neuron-Specific Enolase (NSE) | 0.32 | 0.10 | 0.003 | 0.45 |

The results of the linear regression analysis are explained in Table 3 with significant results for vitreous potassium ($R^2$=0.69) which is a robust predictor of PMI, and CSF NSE ($R^2$=0.56) contributes significantly but explains a lesser part of the variability. In these models, both fluids give valuable information but vitreous humor may be the more reliable fluid to use in estimating PMI when dealing with forensic investigation in Pakistan.

The results show that vitreous potassium levels have a strong and statistically significant correlation with the postmortem interval, which makes them a good parameter to estimate PMI. Although NSE correlates with PMI in CSF as well, its predictive value is much decreased compared to that of CSF markers. This supports the use of a dual fluid approach in forensic investigations where vitreous humor is the primary matrix for PMI estimation, and in cases where additional central nervous system information is needed, CSF markers.

## DISCUSSION

In the present study, we show that vitreous humor and cerebrospinal fluid (CSF) have different biochemical profiles that can be used to estimate postmortem interval (PMI). First, we demonstrate that vitreous potassium levels have a strong positive correlation with PMI ($r = 0.85$; $p < 0.001$), and therefore vitreous potassium levels can serve as a reliable marker in forensic investigations[9]. This observation is substantiated by the regression

model, which explains 72% of the variability in PMI using vitreous potassium. These results are consistent with previous studies that have demonstrated the consistent increase of potassium concentration as a reliable time-since-death indicator[10, 11].

In contrast to the PMI, the CSF markers, especially neuron-specific enolase (NSE), showed a moderate correlation with PMI ($r = 0.68$, $p = 0.002$) and accounted for 45% of the variability. Although the biochemical markers of CSF offer important information (and especially those about the CNS) their predictive value is lower than that of vitreous potassium[12]. While there was a trend toward a correlation of the S100B levels in CSF to PMI ($r = 0.42$, $p = 0.06$), this was not statistically significant, implying that S100B may not be a useful single marker for PMI[13].

Environmental factors, such as ambient temperature and humidity, that were continuously recorded during sample collection are integrated to further improve the robustness of our predictive models. As different climatic conditions are encountered in Pakistan, it is important to account for these variables while estimating PMI to ensure the reliability and reproducibility of PMI estimates[14]. The results highlight the value of adopting a dual-fluid approach in forensic investigations. Despite the unstable nature of vitreous humor and its lower predictive value, vitreous humor is the standard matrix for estimating PMI due to its stability, but CSF analysis may be an advantageous supplement, especially in cases where CNS pathology or trauma may confound traditional vitreous markers[15, 16].

Additionally, our study underscores the necessity to standardize sample collection, handling and analysis methods for reducing the variability and increasing reproducibility of results. This study adopted very stringent inclusion and exclusion criteria in order to study only cases with minimal decomposition and no significant confounding factors, to provide a clearer picture of the biochemical changes postmortem[17, 18].

## CONCLUSION

Finally, the comparison of vitreous humor and CSF biochemical markers shows that vitreous potassium levels are a good and robust biomarker to estimate the postmortem interval in forensic investigations. CSF markers such as neuron-specific enolase (NSE) are valuable, especially regarding central nervous system changes, but have a lower predictive power than vitreous potassium. Utilizing the dual fluid approach along with the consideration of environmental factors is a promising approach to improving the accuracy of PMI estimations in the forensic context of Pakistan. Refinement of these predictive models is warranted with further research with larger sample sizes and more markers to explore the integration of advanced statistical methodologies to improve forensic analysis.

**Funding:** No external funding was received for this study.

**Conflict of Interest:** The authors declare no conflicts of interest related to this study.

**Authors' Contributions:** F.S. and K.M. conceptualized and supervised the study. R.A. and F.T. contributed to data collection, laboratory analysis, and interpretation. Z.I. and A.A. assisted in sample processing, data management, and literature review. All authors contributed to manuscript writing and approved the final version.

**Acknowledgment:** The authors express their gratitude to the Forensic Medicine Departments of Jinnah Hospital and Mayo Hospital, Lahore, for their support and cooperation during sample collection and autopsy procedures. Special thanks to the technical and mortuary staff for their valuable assistance throughout the study.

## REFERENCES

1. Swain R, Kumar A, Sahoo J, Lakshmy R, Gupta S, Bhardwaj D, et al. Estimation of post-mortem interval: A comparison between cerebrospinal fluid and vitreous humour chemistry. Journal of forensic and legal medicine. 2015;36:144-8.
2. Yahia D, Abd El-Hakiem MA. Biochemical analysis of synovial fluid, cerebrospinal fluid and vitreous humor at early postmortem intervals in donkeys. Journal of Advanced Veterinary Research. 2014;4(1):6-11.
3. Garland J, Philcox W, McCarthy S, Kesha K, Lam L, Spark A, et al. Post-mortem biochemistry differences between vitreous humour and cerebrospinal fluid. Australian Journal of Forensic Sciences. 2020;52(5):518-28.
4. Zelentsova EA, Yanshole LV, Melnikov AD, Kudryavtsev IS, Novoselov VP, Tsentalovich YP. Post-mortem changes in metabolomic profiles of human serum, aqueous humor and vitreous humor. Metabolomics. 2020;16:1-10.
5. Focardi M, Lanzilao L, Bonari A, Lazzeretti M, Lorubbio M, Ognibene A, et al. Improvement in estimation of time since death by albumin and potassium concentrations in vitreous humor. Forensic Science International. 2020;314:110393.
6. Boulagnon C, Garnotel R, Fornes P, Gillery P. Post-mortem biochemistry of vitreous humor and glucose metabolism: an update. Clinical Chemistry and Laboratory Medicine (CCLM). 2011;49(8):1265-70.
7. Li W, Chang Y, Cheng Z, Ling J, Han L, Li X, et al. Vitreous humor: A review of biochemical constituents in postmortem interval estimation. Journal of Forensic Science and Medicine. 2018;4(2):85-90.
8. Peyron P-A, Lehmann S, Delaby C, Baccino E, Hirtz C. Biochemical markers of time since death in cerebrospinal fluid: a first step towards "Forensomics". Critical reviews in clinical laboratory sciences. 2019;56(4):274-86.
9. Pigaiani N, Bertaso A, De Palo EF, Bortolotti F, Tagliaro F. Vitreous humor endogenous compounds analysis for post-mortem forensic investigation. Forensic science international. 2020;310:110235.
10. Cordeiro C, Ordóñez-Mayán L, Lendoiro E, Febrero-Bande M, Vieira DN, Muñoz-Barús JI. A reliable method for estimating the postmortem interval from the biochemistry of the vitreous humor, temperature and body weight. Forensic science international. 2019;295:157-68.
11. Garland J, Olds K, Rousseau G, Palmiere C, Ondruschka B, Kesha K, et al. Using vitreous humour and cerebrospinal fluid electrolytes in estimating post-mortem interval-an exploratory study. Australian Journal of Forensic Sciences. 2020;52(6):626-33.
12. McCleskey BC, Dye DW, Davis GG. Review of postmortem interval estimation using vitreous humor: past, present, and future. Academic forensic pathology. 2016;6(1):12-8.
13. Singh G, Sharma D, Pathak D, Dutta S. Comparative Study on Post Mortem Analysis of Sodium and Potassium Levels of Vitreous Humour and Synovial Fluid in Determining Time Since Death. Indian Journal of Forensic Medicine & Toxicology. 2022;16(3):393-8.
14. Tumram NK, Bardale RV, Dongre AP. Postmortem analysis of synovial fluid and vitreous humour for determination of death interval: A comparative study. Forensic science international. 2011;204(1-3):186-90.
15. Koopmanschap DH, Bayat AR, Kubat B, de Bakker HM, Prokop MW, Klein WM. The radiodensity of cerebrospinal fluid and vitreous humor as indicator of the time since death. Forensic Science, Medicine, and Pathology. 2016;12:248-56.
16. Agoro E, Wankasi M, Azuonwu O. The forensic application of vitreous humour biochemistry in postmortem disease diagnosis. Indian Journal of Forensic Medicine & Toxicology. 2017;11(1):193-7.
17. Pérez-Martínez C, Prieto Bonete G, Pérez-Cárceles MD, Luna A. Influence of the nature of death in biochemical analysis of the vitreous humour for the estimation of post-mortem interval. Australian Journal of Forensic Sciences. 2020;52(5):508-17.
18. Kareem SK. Estimation of post-mortem interval by some biochemical changes of vitreous humor. AL-Kindy College Medical Journal. 2015;11(2):69-75.

**This article may be cited as:** Sultana F, Mahmood K, Ali R, Tariq F, Iqbal Z, Abbas A: Comparative Analysis of Postmortem Biochemical Markers in Vitreous Humor and Cerebrospinal Fluid for Estimating Time of Death. Pak J Med Health Sci, 2023; 17(8): 108-110.