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Original Research Article

POSTMORTEM POTASSIUM KINETICS IN VITREOUS HUMOR: A BIOCHEMICAL APPROACH TO TIME SINCE DEATH ESTIMATION IN A FORENSIC COHORT.

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ABSTRACT

Background: Accurate estimation of the postmortem interval (PMI) is critical in forensic investigations, yet conventional physical signs often yield wide margins of error. Biochemical changes in the vitreous humor, particularly shifts in potassium levels, offer a more quantifiable and reliable alternative due to the fluid's anatomical isolation and resistance to early decomposition. The objective is to evaluate the temporal changes in vitreous humor electrolytes—specifically potassium, sodium, and chloride—and assess their utility in estimating time since death.

Materials and Methods: This prospective, one-year study was conducted at the Department of Forensic Medicine, ESIC Medical College Hyderabad, involving 80 medico-legal autopsies with known time of death. Clear vitreous samples were aspirated under sterile conditions and analyzed using ion-selective electrode methods. Correlations between electrolyte concentrations and PMI were assessed using regression modeling.

Results: Potassium levels exhibited a statistically significant linear rise with increasing PMI ($p < 0.001$), forming the basis for a regression model: PMI (hours) = $5.21 \times [K^+] - 29.8$. This model demonstrated strong predictive reliability within the first 48 hours after death. In contrast, sodium and chloride levels showed no meaningful temporal trend or statistical significance ($p > 0.05$).

Conclusion: Vitreous potassium concentration is a dependable postmortem biochemical marker for estimating early PMI. Its integration into forensic protocols may enhance the accuracy of time-of-death estimations, particularly when used alongside other investigative findings.

Keywords: Postmortem interval, Vitreous humor, Potassium, Electrolyte analysis, Forensic time estimation, Autopsy biomarkers.

INTRODUCTION

Estimating the postmortem interval (PMI) remains a cornerstone of forensic investigation, guiding the reconstruction of death timelines and supporting medico-legal conclusions. Conventional methods such as body cooling, rigor mortis, and livor mortis are subject to numerous confounding variables including ambient temperature, body habitus, and environmental exposure, often leading to imprecise estimations beyond the first few hours postmortem.[1,2]

To address these limitations, forensic researchers have increasingly turned toward biochemical analysis of body fluids. Among them, vitreous humor—the transparent gel within the eye—has garnered attention due to its anatomical isolation, delayed decomposition, and resistance to microbial invasion.[3,4] This makes it particularly suitable for the evaluation of biochemical markers that change in a time-dependent fashion after death.

The most extensively studied of these markers is potassium (K^+). Following death, the breakdown of retinal cell membranes leads to a passive diffusion of intracellular potassium into the vitreous chamber, producing a steady rise in concentration over time.[5] This trend has been shown to correlate strongly with PMI, particularly within the first 48 to 72 hours.[6,7] In contrast, sodium (Na^+) and chloride (Cl^-) levels tend to remain relatively stable or decline inconsistently, limiting their reliability as standalone markers.[8]

While earlier studies proposed mathematical models using vitreous potassium for PMI estimation, variations in methodology, climate, population, and cause of death necessitate region-specific validation.[9] Moreover, advances in analytic techniques such as ion-selective electrodes have improved sensitivity and reproducibility, enabling more precise measurements in recent years.[10]

This study was conducted to evaluate the vitreous electrolyte profile in autopsied cases to accurately estimate the PMI.

MATERIALS AND METHODS

This study was a prospective, observational analysis carried out over a one-year period from April 2024 to March 2025 at the Department of Forensic Medicine, ESIC Medical college, Hyderabad. The primary aim was to investigate the postmortem alterations in vitreous humor electrolyte levels and their association with the postmortem interval (PMI). Institutional ethical clearance was obtained prior to the commencement of the study.

A total of 80 medicolegal autopsy cases were included, selected based on the availability of a clearly recorded time of death. Both natural and unnatural deaths were considered, provided the deceased were aged between 15 and 60 years and presented for autopsy within 65 hours after death. Only cases with clear, non-turbid vitreous humor were included to ensure sample quality and analytical reliability.

Exclusion criteria were applied to minimize confounding factors. These included ocular trauma, extensive facial burns, visible putrefaction, known or suspected metabolic or renal disorders, and deaths involving poisoning by agents known to alter serum electrolytes. Additionally, custodial deaths, decomposed bodies, and cases with damaged or absent eyes were excluded from the analysis.

Vitreous humor was collected under aseptic conditions using a 10 mL syringe with a 20-gauge needle. The sampling site was located 5 mm posterior to the limbus. A volume of 1 to 2 mL was aspirated from each eye and pooled when feasible. To preserve the cosmetic appearance, an equal amount of normal saline was injected into the eyeball post-aspiration. All samples were labeled and promptly transported under cold chain to the biochemistry laboratory for analysis.

Samples were centrifuged at 3000 rpm for 10 minutes to eliminate cellular debris, and the supernatant was analyzed for potassium (K^+), sodium (Na^+), and chloride (Cl^-) using an ion-selective electrode system. The equipment was routinely calibrated using manufacturer-supplied standards, and quality control measures were followed throughout the process. Electrolyte concentrations were measured in millimoles per liter (mmol/L).

Relevant case details such as age, sex, cause of death, and environmental conditions were recorded. The postmortem interval was calculated as the time between death and sample collection. Potassium levels were the main variable studied for their correlation with PMI, while sodium and chloride were evaluated as secondary markers.

Data were entered into SPSS software (25.0) for statistical analysis. Quantitative variables were expressed as mean ± standard deviation. Correlation between vitreous potassium and PMI was tested using Pearson's correlation coefficient. A linear regression equation was derived to estimate PMI from potassium concentration. One-way analysis of variance (ANOVA) was used to assess group differences in sodium and chloride levels across various PMI intervals. A p-value less than 0.05 was considered statistically significant.

RESULTS

A total of 80 postmortem cases were included in the study. The age of the deceased ranged from 16 to 60 years, with a mean age of 37.8 years. Males constituted the majority of the study population, accounting for 58 cases (72.5%), while females comprised 22 cases (27.5%). Most individuals were between 21 and 40 years of age, representing 51.3% of the total sample.

The postmortem interval (PMI) among the included cases ranged from 1.5 hours to 64 hours, with a mean PMI of 20.1 ± 12.7 hours. For analytical clarity, cases were grouped into three intervals: less than 12 hours, 12 to 24 hours, and more than 24 hours. The majority (34 cases, 42.5%) had a PMI exceeding 24 hours.

Table 1: Age and sex distribution of cases (N = 80)

Age group (years)	Male	Female	Total
< 20	4	2	6
21–40	30	11	41
41–60	22	8	30
> 60	2	1	3
Total	58	22	80

Most deaths (n = 33, 41.3%) resulted from road traffic accidents, followed by poisoning (n = 14, 17.5%) and burns (n = 10, 12.5%). Other causes included hanging, electrocution, and natural deaths such as myocardial infarction. Snakebite and head injury cases were infrequent.

Table 2: Cause of death distribution

Cause of Death	Number of Cases	Percentage (%)
Road Traffic Accidents	33	41.3
Poisoning	14	17.5
Burns	10	12.5
Hanging	8	10.0
Electrocution	4	5.0
Natural Death	5	6.3
Snakebite	3	3.8
Head Injury	3	3.8
Total	80	100.0

Analysis of electrolyte levels revealed that potassium concentration in vitreous humor increased steadily with longer PMI. The mean potassium values were 6.98 ± 0.64 mmol/L for PMI <12 hours, 9.26 ± 0.78 mmol/L for PMI between 12–24 hours, and 12.14 ± 1.92 mmol/L for PMI >24 hours. This increase was statistically significant ($p < 0.001$). In contrast, sodium and chloride levels demonstrated only mild fluctuations across PMI groups, with no statistically significant differences.

Table 3: Vitreous electrolyte levels by PMI group

PMI Group	Mean Potassium (mmol/L)	Mean Sodium (mmol/L)	Mean Chloride (mmol/L)
< 12 hours (n = 22)	6.98 ± 0.64	145.2 ± 10.1	118.4 ± 9.5
12–24 hours (n = 24)	9.26 ± 0.78	146.9 ± 11.8	120.7 ± 10.3
> 24 hours (n = 34)	12.14 ± 1.92	144.5 ± 14.7	122.1 ± 12.4
p-value	< 0.001	0.618	0.489

A strong positive correlation was observed between potassium levels and PMI (Pearson's $r = 0.88$, $p < 0.001$). A linear regression analysis yielded the following equation for estimating PMI based on vitreous potassium levels:

$$\text{PMI (hours)} = 5.21 \times [K^+] - 29.8$$

This model demonstrated good fit with an R^2 value of 0.78. Sodium and chloride did not show any statistically meaningful correlation with PMI ($r = -0.11$ and $r = -0.08$, respectively; both $p > 0.05$).

Table 4. Correlation of vitreous electrolytes with PMI

Electrolyte	Pearson's r	p-value	Regression Equation	R^2
Potassium	0.88	<0.001	PMI = 5.21 × $[K^+]$ – 29.8	0.78
Sodium	–0.11	0.312	Not significant	–
Chloride	–0.08	0.407	Not significant	–

These findings support the utility of potassium concentration in vitreous humor as a reliable biochemical indicator for estimating time since death, especially within the first 48 hours. Sodium and chloride levels, while relatively stable, lack sufficient discriminatory value for PMI determination.

DISCUSSION

Accurate estimation of the postmortem interval (PMI) remains a persistent challenge in forensic practice, particularly when physical findings are unreliable due to delayed discovery or environmental interference. In this study, a progressive and statistically significant rise in vitreous potassium concentration was observed with increasing time since death, validating its utility as a biochemical tool for estimating PMI.

The linear regression formula derived from this study, PMI = 5.21 × $[K^+]$ – 29.8, demonstrated strong correlation ($r = 0.88$) and a high coefficient of determination ($R^2 = 0.78$), consistent with earlier findings in literature. James et al,[6] and Rognum et al,[7] similarly reported high correlation coefficients, reinforcing potassium's reliability as a marker during early postmortem intervals.

The rise in vitreous potassium can be attributed to autolytic cellular breakdown following death, particularly in the retinal and glial cells. This results in leakage of intracellular potassium into the extracellular vitreous space as membrane integrity declines.[5] Given its anatomical isolation and slower decomposition, the vitreous body serves as a relatively stable environment for postmortem biochemical assessment.[3,4]

In contrast, sodium and chloride levels in this study did not exhibit statistically significant variation across the PMI groups. This finding is in agreement with Zhu et al,[8] and Madea et al,[2] who concluded that while these ions may show mild fluctuations, they lack consistency and utility for PMI estimation. Their changes are more likely influenced by agonal events, dehydration, or individual variability rather than the passage of time itself.

A strength of the current study was the strict inclusion of only cases with known time of death and minimal risk of pre-mortem electrolyte imbalance. The use of ion-selective electrode methodology,[10] further ensured precision and reproducibility of the results. However, some limitations must be acknowledged. Factors such as ambient temperature and terminal illness were not incorporated into the regression model. Previous studies, including Komuro and

Oshiro,[11] have highlighted that elevated ambient temperatures can accelerate potassium diffusion, thereby influencing PMI estimations.

Additionally, the plateauing of potassium values observed beyond 48–60 hours likely reflects the achievement of equilibrium between intracellular and extracellular compartments. This has also been reported in canine studies and extended PMI evaluations.[12] Therefore, the reliability of vitreous potassium for PMI estimation may be best confined to the first 48 hours postmortem.

Compared to earlier models, the regression slope in our study (5.21) is closely aligned with the model proposed by Madea et al. ($5.26 \times [K^+] - 30.9$),[2] and slightly flatter than the equation proposed by Sturner ($7.14 \times [K^+] - 39.1$).[13] These small differences may reflect regional and methodological variations. It is essential to emphasize the importance of local validation of such formulas before implementation in forensic protocols.

CONCLUSION

This prospective study reinforces the value of vitreous humor potassium estimation as a reliable, quantifiable tool for determining postmortem interval during the early stages after death. A strong linear correlation was observed between potassium concentration and PMI, with the derived regression model demonstrating robust predictive accuracy. In contrast, sodium and chloride levels showed minimal variation and lacked significant association with time since death. Given its stability, ease of sampling, and reproducibility, vitreous potassium analysis should be considered a complementary method in routine forensic practice, especially when conventional indicators are unreliable. Future studies incorporating ambient temperature, cause of death, and other metabolic variables may further refine region-specific models for broader application.

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