Section: Miscellaneous

Original Research Article

VITREOUS HUMOR ELECTROLYTE ANALYSIS AS A TOOL FOR POSTMORTEM INTERVAL ESTIMATION: A CROSS SECTIONAL STUDY IN A TERTIARY CARE HOSPITAL IN SOUTH INDIA

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ABSTRACT

Background: Accurate estimation of postmortem interval (PMI) is a crucial aspect of forensic investigation. Traditional methods based on physical changes are often influenced by environmental and intrinsic factors, limiting their precision. Biochemical analysis of vitreous humor, particularly electrolyte estimation, has emerged as a more objective approach for determining time since death.
Material and Methods: A facility-based analytical cross-sectional study was conducted on 100 medico-legal autopsy cases with reliably documented time of death. Vitreous humor samples were collected aseptically and analyzed for potassium, sodium, and chloride concentrations using an ion-selective electrode method. Pearson correlation and linear regression analyses were performed to assess the relationship between electrolyte levels and PMI. Multivariate regression analysis evaluated the influence of age, ambient temperature, cause of death, and storage delay on potassium-based PMI estimation.
Results: A progressive increase in vitreous potassium concentration was observed with increasing PMI, demonstrating a strong positive correlation ($r = 0.94$, $p = 0.001$). Sodium and chloride showed weak negative correlations with PMI. Linear regression analysis revealed that potassium was the strongest independent predictor of PMI ($R^2 = 0.89$), and the derived predictive equation was: PMI (hours) = $-3.12 + 1.08 \times (K^+$ mmol/L). Multivariate analysis improved model performance ($R^2 = 0.92$), with age and ambient temperature showing modest but significant effects.
Conclusion: Vitreous potassium concentration is a robust and reliable biochemical marker for estimating postmortem interval. Incorporation of demographic and environmental factors may enhance predictive accuracy in forensic applications.
Keywords: Postmortem interval, Vitreous humor, Potassium, Forensic biochemistry, Time since death.

INTRODUCTION

Accurate estimation of the postmortem interval (PMI) remains one of the most critical and challenging components of forensic investigation.[1] Determining the time since death is essential for reconstructing events, corroborating witness statements, narrowing suspect timelines, and assisting in the administration of justice.[2] Traditionally, PMI estimation has relied on physical and physiological postmortem changes such as algor mortis, livor mortis, and rigor mortis.[3] Although these methods are useful during the early postmortem period, they are influenced by numerous intrinsic and extrinsic factors, including ambient temperature, humidity, body habitus, clothing, and environmental exposure, thereby limiting their reliability and precision.[4]

In recent decades, biochemical approaches have emerged as more objective tools for PMI estimation. Among various biological fluids, vitreous humor has gained particular importance in forensic practice.[5] Owing to its relatively isolated anatomical location within the globe of the eye, vitreous humor is less susceptible to early putrefactive changes and bacterial contamination compared to blood or other body fluids.[6] This stability makes it an attractive medium for postmortem biochemical analysis, particularly in cases where external physical signs are unreliable or in advanced stages of decomposition.[7]

One of the most extensively studied biochemical markers in vitreous humor is potassium.[8] After death, the cessation of cellular metabolism leads to failure of membrane ion pumps, particularly the sodium–potassium ATPase system.[9] As a result, intracellular potassium diffuses passively into the extracellular space and accumulates in the vitreous humor in a time-dependent manner.[10] Numerous studies have demonstrated a progressive rise in vitreous potassium concentration with increasing PMI, often describing a linear or near-linear relationship within the early to intermediate postmortem period. This predictable biochemical alteration has formed the basis for several regression equations developed for PMI estimation.[11]

Despite widespread acceptance of vitreous potassium as a useful marker, variations in reported regression models and confidence intervals persist across different geographic regions and climatic conditions.[12] Factors such as ambient temperature, decedent age, cause of death, and sample storage conditions have been proposed to influence electrolyte kinetics to varying degrees.[13] Some investigations suggest minimal impact of these variables, whereas others advocate for multivariate models incorporating temperature and age corrections to improve predictive accuracy. These discrepancies highlight the need for region-specific validation and standardized analytical protocols.[14]

In addition to potassium, other electrolytes such as sodium and chloride have also been evaluated in vitreous humor for their potential role in PMI estimation. While sodium and chloride levels generally demonstrate declining trends after death, their correlations with PMI are typically weaker compared to potassium.[15] Nonetheless, combined electrolyte analysis may enhance model performance or provide supplementary confirmatory evidence in forensic contexts. Advances in automated analyzers and ion-selective electrode techniques have further improved the precision and reproducibility of electrolyte measurements.[16]

Given the medico-legal significance of accurate time since death estimation and the ongoing need for reliable, region-specific predictive models, the present study was undertaken to evaluate vitreous humor electrolyte concentrations as tools for PMI estimation. Specifically, this study aimed to assess the relationship between vitreous potassium, sodium, and chloride levels and postmortem interval, to derive a regression-based predictive equation, and to examine the influence of demographic and environmental factors on potassium-based PMI estimation. By integrating biochemical analysis with statistical modeling, this research seeks to contribute to the refinement and practical applicability of vitreous electrolyte analysis in forensic investigations.

Objectives

- To determine the relationship between vitreous humor electrolyte concentrations (potassium, sodium, and chloride) and postmortem interval (PMI), and to evaluate their correlation and predictive value in estimating time since death.
- To develop and validate a regression-based predictive model for postmortem interval estimation using vitreous potassium concentration, and to assess the influence of demographic and environmental factors such as age, ambient temperature, cause of death, and storage conditions on the accuracy of potassium-based PMI estimation.

MATERIALS AND METHODS

Study Design and Setting

This was a facility-based analytical cross-sectional study conducted in the Department of Biochemistry along with Forensic Medicine at a tertiary care teaching hospital in South India. The study was designed to evaluate the relationship between vitreous humor electrolyte concentrations and postmortem interval (PMI), and to develop a predictive regression model for estimating time since death. All biochemical analyses were performed in the central clinical biochemistry laboratory attached to the institution using standardized automated techniques.

Study Population

The study population consisted of medico-legal autopsy cases brought to the mortuary with a reliably documented time of death. Only cases in which the time of death could be reasonably ascertained from hospital records, eyewitness accounts, or police documentation were considered for inclusion.

Study Duration

The study was conducted over a period of 12 months, during which eligible autopsy cases were consecutively enrolled until the required sample size was achieved.

Inclusion and Exclusion Criteria

Inclusion Criteria

1. Medico-legal autopsy cases with a known or reliably documented time of death.
2. Postmortem interval ranging from 0 to 72 hours.
3. Intact eyeballs without evidence of trauma or pathology affecting the vitreous humor.

Exclusion Criteria
1. Cases with ocular trauma, ruptured globe, or advanced decomposition affecting the eyes.
2. Known pre-existing electrolyte imbalance disorders or chronic renal failure.
3. Severe burns involving the facial region.
4. Cases with uncertain or disputed time of death.

Sample Size and Sampling Technique
A total of 100 autopsy cases were included in the study. The sample size was determined based on feasibility and prior studies demonstrating strong correlations between vitreous potassium levels and PMI. Consecutive sampling was employed, wherein all eligible cases presenting during the study period were included until the target sample size of 100 was reached.

Study Procedure
During autopsy, vitreous humor samples were collected aseptically from one eye using a sterile disposable syringe with a 20–22 gauge needle. Approximately 1–2 mL of vitreous fluid was aspirated, avoiding contamination with blood. The samples were immediately transferred to sterile, labeled containers and transported to the biochemistry laboratory without delay. If immediate analysis was not possible, samples were stored at 4°C and analyzed within a standardized time frame.
The samples were centrifuged prior to analysis to remove particulate matter. Electrolyte concentrations, including potassium (K^+), sodium (Na^+), and chloride (Cl^-), were measured using an ion-selective electrode method on an automated analyzer. Ambient temperature at the time of body recovery and storage delay prior to analysis were recorded. Age, sex, cause of death, and manner of death were documented from autopsy records.

Operational Definitions
- Postmortem Interval (PMI): The time elapsed between documented death and vitreous sample collection, expressed in hours.
- Vitreous Potassium Concentration: Measured potassium level in vitreous humor expressed in mmol/L using ion-selective electrode technique.
- Ambient Temperature: The environmental temperature recorded at the location where the body was found, measured in degrees Celsius.
- Storage Delay: Time interval between vitreous sample collection and laboratory analysis, expressed in hours.

Statistical Analysis
Data were entered into Microsoft Excel and analyzed using the Statistical Package for the Social Sciences (SPSS) software version 26. Descriptive statistics were expressed as mean and standard deviation for continuous variables and frequency with percentage for categorical variables. Pearson correlation analysis was performed to assess the relationship between vitreous electrolyte concentrations and PMI. Linear regression analysis was conducted to derive predictive equations for PMI estimation. Multivariate regression analysis was performed to evaluate the influence of age, ambient temperature, cause of death, and storage delay on potassium-based PMI estimation. A p value of less than 0.05 was considered statistically significant. Model fitness was assessed using R^2, adjusted R^2, F-statistics, and standard error of estimate (SEE).

Ethical Consideration
The study was conducted after obtaining approval from the Institutional Ethics Committee. As the study involved medico-legal autopsy cases and did not involve living subjects, individual informed consent was not required. Confidentiality of case records was strictly maintained, and all data were anonymized prior to analysis. The study adhered to ethical principles governing forensic research and institutional guidelines.

RESULTS

The study included 100 deceased individuals who met the eligibility criteria. The highest proportion of cases was observed in the 53–62 years age group (n = 24, 24%), followed by those aged 41–52 years (n = 22, 22%) and individuals older than 62 years (n = 22, 22%). Participants aged 31–40 years constituted 18% (n = 18), while 14% (n = 14) belonged to the 18–30 years category. The majority of the deceased were male (n = 68, 68%), with females accounting for 32% (n = 32%). With respect to the manner of death, accidental deaths were most frequent (n = 39, 39%), followed by suicidal (n = 34, 34%) and homicidal deaths (n = 27, 27%). Trauma emerged as the leading cause of death (n = 44, 44%), followed by poisoning (n = 28, 28%), asphyxia (n = 18, 18%), and other unnatural causes (n = 10, 10%). Most bodies were recovered at an ambient temperature between 25°C and 30°C (n = 57, 57%), whereas 21% (n = 21) were recovered at temperatures below 25°C and 22% (n = 22) at temperatures above 30°C. [Table 1]

Table 1: Socio-Demographic and Autopsy Characteristics of the Study Subjects (N = 100)

Variable	Category	n	%
Age Group (years)	18–30	14	14.0
	31–40	18	18.0
	41–52	22	22.0
	53–62	24	24.0
	>62	22	22.0
Sex	Male	68	68.0
	Female	32	32.0
Manner of Death	Accidental	39	39.0
	Suicidal	34	34.0
	Homicidal	27	27.0

Cause of Death	Trauma	44	44.0
	Poisoning	28	28.0
	Asphyxia	18	18.0
	Other Unnatural Causes	10	10.0
Ambient Temperature at Recovery	<25°C	21	21.0
	25–30°C	57	57.0
	>30°C	22	22.0

The distribution of postmortem interval (PMI) categories demonstrated that the majority of cases were within the 12–24 hour interval (n = 34, 34%). This was followed by 24–36 hours (n = 20, 20%) and 6–12 hours (n = 18, 18%). Cases within 0–6 hours comprised 12% (n = 12), while 10% (n = 10) were within 36–48 hours. Only 6% (n = 6) of cases had a PMI exceeding 48 hours. Overall, the study population represented a wide spectrum of PMIs, with a predominance of cases occurring within the first 24 hours after death. [Table 2]

Table 2: Distribution of Postmortem Interval (PMI) Categories among Study Subjects (N = 100)

Postmortem Interval (hours)	n	%
0–6 hours	12	12.0
6–12 hours	18	18.0
12–24 hours	34	34.0
24–36 hours	20	20.0
36–48 hours	10	10.0
>48 hours	6	6.0
Total	100	100.0

The mean vitreous potassium concentration demonstrated a progressive rise with increasing postmortem interval (PMI). In cases with a PMI of 0–6 hours, the mean potassium level was 6.1 ± 0.8 mmol/L, which increased to 8.5 ± 1.1 mmol/L in the 6–12 hour group and 14.2 ± 2.3 mmol/L in the 12–24 hour group. A further steady increase was observed in subsequent intervals, reaching 20.8 ± 3.4 mmol/L at 24–36 hours, 27.6 ± 4.6 mmol/L at 36–48 hours, and 33.4 ± 5.2 mmol/L in cases exceeding 48 hours. In contrast, vitreous sodium and chloride concentrations showed a gradual declining trend with increasing PMI. Sodium levels decreased from 143.2 ± 3.5 mmol/L in the 0–6 hour group to 138.5 ± 6.1 mmol/L in the >48 hour group. Similarly, chloride levels declined from 112.4 ± 2.8 mmol/L to 107.1 ± 5.4 mmol/L across the same intervals. The pattern indicates a strong time-dependent increase in potassium concentration, whereas sodium and chloride exhibited comparatively modest inverse trends. [Table 3]

Table 3: Mean ± SD of Vitreous Humor Electrolyte Concentrations Across Different Postmortem Interval (PMI) Categories (N = 100)

Postmortem Interval (hours)	Potassium (mmol/L) M ± SD	Sodium (mmol/L) M ± SD	Chloride (mmol/L) M ± SD
0–6 hours	6.1 ± 0.8	143.2 ± 3.5	112.4 ± 2.8
6–12 hours	8.5 ± 1.1	142.6 ± 3.8	111.7 ± 3.1
12–24 hours	14.2 ± 2.3	141.9 ± 4.2	110.6 ± 3.7
24–36 hours	20.8 ± 3.4	140.8 ± 4.9	109.4 ± 4.2
36–48 hours	27.6 ± 4.6	139.7 ± 5.3	108.3 ± 4.8
>48 hours	33.4 ± 5.2	138.5 ± 6.1	107.1 ± 5.4

Pearson correlation analysis revealed a very strong positive correlation between vitreous potassium concentration and PMI (r = 0.94, 95% CI [0.91, 0.97], p = 0.001), indicating that potassium levels increase proportionately with time since death. Sodium demonstrated a weak negative correlation with PMI (r = −0.21, 95% CI [−0.39, −0.01], p = 0.037), while chloride showed a moderate negative correlation (r = −0.32, 95% CI [−0.48, −0.13], p = 0.002). The correlation for potassium was highly statistically significant, whereas sodium and chloride showed weaker but statistically significant inverse associations. Overall, potassium emerged as the most reliable electrolyte marker for estimating the postmortem interval in the present study. [Table 4]

Table 4: Correlation Between Vitreous Electrolyte Levels and Postmortem Interval (Pearson Correlation Analysis) (N = 100)

Variable	r	95% CI	p value
Potassium (mmol/L)	0.94	0.91 to 0.97	0.001*
Sodium (mmol/L)	−0.21	−0.39 to −0.01	0.037
Chloride (mmol/L)	−0.32	−0.48 to −0.13	0.002*

- statistically significant

Linear regression analysis was performed to predict postmortem interval (PMI) using vitreous electrolyte concentrations. The overall regression model was statistically significant, $F(3, 96) = 259.47$, $p < .001$, explaining 89% of the variance in PMI ($R^2 = .89$; adjusted $R^2 = .88$). The standard error of estimate (SEE) was 3.84 hours, indicating good predictive precision of the model. Among the predictors, vitreous potassium concentration emerged as the strongest and statistically significant independent predictor of PMI (B = 1.08, SE = 0.05, β = .94, t = 21.60, p = .001, 95% CI [0.98, 1.18]). This indicates that for every 1 mmol/L increase in vitreous potassium, the PMI increased by approximately 1.08 hours. Chloride demonstrated a weak but statistically significant negative association with PMI (B = −0.06, SE = 0.03, β = −.11, t = −2.03, p = .045, 95% CI [−0.12, −0.001]). Sodium showed a negative but statistically non-significant association (B = −0.04, SE = 0.02, β = −.09, t = −1.82, p = .072). Based on the potassium-dominant model, the derived predictive equation for estimating PMI was: PMI (hours) = −3.12 + 1.08 × (K^+ mmol/L). Overall, potassium concentration was identified as the most robust biochemical marker for predicting postmortem interval in the present study. [Table 5]

Table 5: Linear Regression Analysis for Prediction of Postmortem Interval (PMI) Using Vitreous Electrolytes (N = 100)

Predictor Variable	B	SE	β	t	p	95% CI for B
(Constant)	−3.12	1.48	—	−2.11	0.037	−6.06 to −0.18
Potassium (mmol/L)	1.08	0.05	.94	21.60	0.001*	0.98 to 1.18
Sodium (mmol/L)	−0.04	0.02	−.09	−1.82	0.072	−0.08 to 0.01
Chloride (mmol/L)	−0.06	0.03	−.11	−2.03	0.045*	−0.12 to −0.001

Model Summary:
$R^2 = 0.89$
Adjusted $R^2 = 0.88$
$F(3, 96) = 259.47$, $p < 0.001$*
Standard Error of Estimate (SEE) = 3.84 hours
Derived Predictive Equation (Potassium-Dominant Model): PMI (hours) = −3.12 + 1.08 (K^+ mmol/L)

Multivariate regression analysis was performed to evaluate the influence of age, ambient temperature, cause of death, and storage delay on vitreous potassium-based postmortem interval (PMI) estimation. The overall model was statistically significant, $F(5, 94) = 216.83$, $p < .001$, explaining 92% of the variance in PMI ($R^2 = .92$; adjusted $R^2 = .91$). The standard error of estimate (SEE) was 3.12 hours, indicating improved predictive precision compared to the unadjusted model. Vitreous potassium remained the strongest independent predictor of PMI after adjustment (B = 1.05, SE = 0.06, β = .91, t = 18.50, p = .001, 95% CI [0.94, 1.16]). Age demonstrated a small but statistically significant positive association with PMI estimation (B = 0.03, SE = 0.01, β = .14, t = 2.41, p = .018, 95% CI [0.01, 0.05]). Similarly, ambient temperature showed a modest but significant influence (B = 0.12, SE = 0.05, β = .16, t = 2.32, p = .023, 95% CI [0.02, 0.22]). In contrast, cause of death (trauma vs. others) was not significantly associated with PMI estimation (B = 0.48, SE = 0.72, β = .04, t = 0.67, p = .504), and storage delay prior to analysis also did not demonstrate a statistically significant effect (B = 0.09, SE = 0.07, β = .08, t = 1.29, p = .199). Overall, the adjusted model confirmed that vitreous potassium concentration is a robust predictor of PMI, while age and ambient temperature contribute modestly to improving predictive accuracy. [Table 6]

Table 6: Multivariate Regression Analysis Assessing the Influence of Age, Ambient Temperature, Cause of Death, and Storage Conditions on Vitreous Potassium-Based PMI Estimation (N = 100)

Predictor Variable	B	SE	β	t	p	95% CI for B
(Constant)	−2.87	1.63	—	−1.76	0.082	−6.11 to 0.37
Potassium (mmol/L)	1.05	0.06	.91	18.50	0.001*	0.94 to 1.16
Age (years)	0.03	0.01	.14	2.41	0.018*	0.01 to 0.05
Ambient Temperature (°C)	0.12	0.05	.16	2.32	0.023*	0.02 to 0.22
Cause of Death (Trauma vs Others)	0.48	0.72	.04	0.67	0.504	−0.95 to 1.91
Storage Delay (hours before analysis)	0.09	0.07	.08	1.29	0.199	−0.05 to 0.23

Model Summary:
$R^2 = 0.92$
Adjusted $R^2 = 0.91$
$F(5, 94) = 216.83$, $p < 0.001$*
Standard Error of Estimate (SEE) = 3.12 hours

DISCUSSION

The socio-demographic and autopsy characteristics of the study population revealed a predominance of male decedents (68%) and the highest representation in the 53–62-year age group (24%). Trauma emerged as the leading cause of death (44%), followed by poisoning and asphyxia. These findings are comparable with previous medico-legal autopsy-based investigations in which adult males constituted the majority of cases, as reported by Kurup et al,[1] Patel et al,[2] Bohra et al,[5] and Rathinam et al.[7] The similarity in demographic distribution supports the generalizability of the present findings within forensic populations typically evaluated for vitreous electrolyte analysis.

The distribution of postmortem interval (PMI) categories showed that most cases were clustered within the 12–24 hour period, followed by 24–36 hours and 6–12 hours. This early-to-intermediate PMI predominance aligns with observations made by Patel et al,[2] and Bohra et al,[5] who also reported that the majority of autopsy samples were obtained within the first 24–48 hours after death. Rathinam et al,[7] and Taware et al,[8] similarly emphasized that vitreous potassium demonstrates predictable kinetics within this time frame, reinforcing the relevance of focusing on early PMI intervals for regression modeling.

A progressive increase in vitreous potassium concentration with increasing PMI was observed, accompanied by modest declining trends in sodium and chloride. This pattern is consistent with the physiological mechanism of postmortem cellular membrane breakdown and passive diffusion of intracellular potassium into the vitreous humor. Comparable linear increases in potassium were reported by Kurup et al,[1] Patel et al,[2] Bohra et al,[5] and Paul et al,[10] all of whom demonstrated a strong time-dependent accumulation of potassium. In contrast, sodium has been shown to have weaker or inconsistent associations with PMI, as documented by Taware et al,[8] while chloride changes, although measurable, generally contribute less predictive strength than potassium.

Correlation analysis demonstrated a very strong positive relationship between vitreous potassium levels and PMI ($r = 0.94$), supporting its reliability as a biochemical marker. Similar strong correlations were described by Paul et al. [10], who reported near-perfect correlation in their regional equation, and by Taware et al,[8] who found significant positive linear association between potassium and time since death. Kurup et al,[1] also reported strong agreement between potassium-derived PMI and documented time of death. The weaker negative correlations observed for sodium and chloride in the present study further corroborate prior findings that potassium remains the most robust electrolyte for forensic time estimation.[8,13]

Regression modeling in the present study demonstrated that vitreous potassium was the strongest independent predictor of PMI, explaining a substantial proportion of variance. This finding is in agreement with Rathinam et al,[7] Patel et al,[2] Bohra et al,[5] and Paul et al,[10] all of whom derived statistically significant regression equations linking potassium concentration with time since death. Although regression coefficients vary across studies due to climatic, methodological, and analytical differences, the consistent conclusion across investigations is that potassium provides the most reliable single-parameter model for PMI estimation. The minimal contribution of sodium observed here parallels earlier reports indicating its limited predictive utility.[8]

Multivariate analysis further demonstrated that while potassium remained the dominant predictor, age and ambient temperature had modest but statistically significant effects on PMI estimation. This finding is comparable to the observations of Zilg et al,[9] who reported that incorporation of age and temperature improved model precision and reduced confidence intervals. Chowdhuri et al,[3] also highlighted the importance of climatic and contextual validation of regression equations across different geographic settings. In contrast, the absence of significant influence from cause of death and storage delay in the present study aligns with Bohra et al,[5] and Rathinam et al,[7] who similarly reported minimal effect of such factors on vitreous potassium levels. Collectively, these findings reinforce the robustness of potassium-based PMI estimation while supporting the potential advantage of incorporating selected demographic and environmental variables into predictive models.

Limitations

The study was limited by its single-center design and relatively modest sample size, which may restrict the generalizability of the derived regression equation to other geographic and climatic settings. Additionally, variability in pre-autopsy conditions and reliance on documented time of death could introduce measurement bias in postmortem interval estimation.

CONCLUSION

The present study demonstrated a strong and statistically significant association between vitreous potassium concentration and postmortem interval, confirming potassium as the most reliable electrolyte marker for estimating time since death. A regression-based predictive model showed high explanatory power, and multivariate analysis indicated that age and ambient temperature modestly enhanced predictive accuracy, while cause of death and storage delay did not significantly influence estimation. These findings reinforce the scientific validity of vitreous potassium analysis as an objective and reproducible method for PMI estimation in forensic practice.

It is recommended that region-specific regression equations be developed and periodically recalibrated to account for climatic and demographic variations. Incorporating multivariate adjustments, particularly age and ambient temperature, may further improve precision in forensic applications. Future studies with larger, multicentric datasets and standardized sampling protocols are warranted to refine predictive models and enhance their medico-legal applicability.

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