Original Article

Comparative Analysis of Cerebrospinal Fluid and Synovial Fluid Electrolytes for Estimation of Time Since Death: A Cross-Sectional Autopsy Study

Tauheed Anwar[1], Anoop Kumar Verma[2], Mousami Singh[3], Raja Rupani[3], Sangeeta Kumari[4], Shiuli[4], Md. Kaleem Ahmad[5]

[1]Senior Resident, Department of Forensic Medicine & Toxicology, King George's Medical University, Lucknow, Uttar Pradesh, India. .[2]Professor & Head, Department of Forensic Medicine & Toxicology, King George's Medical University, Lucknow, Uttar Pradesh, India [3]Professor, Department of Forensic Medicine & Toxicology, King George's Medical University, Lucknow, Uttar Pradesh, India. [4]Additional Professor, Department of Forensic Medicine & Toxicology, King George's Medical University, Lucknow, Uttar Pradesh, India. [5]Professor, Department of Biochemistry, King George's Medical University, Lucknow, Uttar Pradesh, India.

Abstract

Background: Accurate estimation of the postmortem interval (PMI) remains a major challenge in forensic practice, as conventional methods are influenced by environmental and individual factors. Thanatochemistry provides a more objective approach through predictable biochemical changes after death. Synovial fluid (SF) and cerebrospinal fluid (CSF), owing to their relative anatomical isolation, may serve as alternative matrices to vitreous humour. Postmortem disruption of cellular membranes causes measurable alterations in sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}). Comparative evidence regarding these electrolytes in SF and CSF remains limited. **Material and Methods:** This was a cross-sectional comparative study. A total of 100 medico-legal autopsy cases with known time since death (up to 24 hours) were included in this prospective study. CSF was collected via lumbar puncture, and SF was aspirated from the knee joint. Electrolyte levels (Na^+, K^+, Ca^{2+}) were measured using ion-selective electrode methods. Cases were grouped based on PMI: 0–6, 7–12, 13–18, and 19–24 hours. Statistical analysis was performed using Jamovi 2.3.37 with descriptive and inferential statistics, applying Pearson correlation and linear regression models to identify relationships. **Results:** This study evaluated postmortem sodium, potassium, and calcium concentrations in cerebrospinal fluid (CSF) and synovial fluid (SF) during the first 24 hours after death. SF potassium showed the strongest correlation with exact time since death (TSD; r=0.467), followed by CSF potassium (r=0.373), whereas CSF calcium declined with increasing TSD (r=−0.274). The SF electrolyte model explained more variation than the CSF model, while the combined six-electrolyte model provided the best fit (R^2=0.414; adjusted R^2=0.376). **Conclusion:** Synovial-fluid potassium showed the strongest association with time since death and performed better than cerebrospinal-fluid potassium during the first 24 hours. Combining electrolytes from both fluids improved predictive performance, but the remaining error limits standalone forensic application. These estimates should therefore be interpreted alongside conventional postmortem findings, with external validation required before routine use.

Keywords: Autopsy, Cerebrospinal fluid, Synovial fluid, Electrolytes, Postmortem Interval

Received: 02 July 2026 **Revised: 19 July 2026** **Accepted: 04 August 2026** **Published: 22 August 2026**

Introduction

Estimation of the postmortem interval (PMI) remains one of the most challenging yet crucial responsibilities of the forensic pathologist, carrying significant implications in both criminal investigations and civil disputes.[1] Conventional methods based on postmortem changes—such as rigor mortis, hypostasis, algor mortis, and decomposition—are inherently subjective and markedly influenced by environmental and individual factors, resulting in wide margins of error as the postmortem interval increases.[2]

To overcome these limitations, forensic science has progressively embraced thanatochemistry, which is based on predictable biochemical alterations occurring after death and provides quantitative measurements that may assist in determining the postmortem interval.[3,4] Among the various biological fluids studied, compartmental fluids have gained prominence because of their relative isolation from environmental influences and delayed putrefactive changes. Although vitreous humour has been extensively investigated and widely utilized for PMI estimation, its applicability may be restricted in cases involving ocular trauma, severe craniofacial injuries, or advanced decomposition, thereby necessitating exploration of alternative biological matrices. Synovial fluid (SF) and cerebrospinal fluid (CSF) represent promising yet comparatively underutilized matrices for PMI estimation. Synovial fluid, enclosed within joint spaces, is relatively protected from external contamination and demonstrates gradual

Address for correspondence: Dr. Tauheed Anwar, Senior Resident, Department of Forensic Medicine & Toxicology, King George's Medical University, Lucknow, Uttar Pradesh, India.
E-mail: anwertauheed11@gmail.com

DOI:
10.21276/amit.2026.v13.i2.974

How to cite this article: Anwar T, Verma AK, Singh M, Rupani R, Kumari S, Shiuli, Ahmad MK. Comparative Analysis of Cerebrospinal Fluid and Synovial Fluid Electrolytes for Estimation of Time Since Death: A Cross-Sectional Autopsy Study. Acta Med Int. 2026:13(2):1873-1879.

postmortem biochemical alterations, particularly in electrolyte concentrations.[5] Similarly, CSF reflects postmortem cellular membrane failure and ionic redistribution; however, its rapid and occasionally variable biochemical changes necessitate careful interpretation.

Electrolytes such as sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}) play fundamental roles in maintaining cellular homeostasis during life. Following death, cessation of active membrane transport, disruption of ionic gradients, and progressive autolysis result in predictable shifts in electrolyte concentrations. Potassium concentration typically increases due to intracellular leakage secondary to membrane breakdown, while sodium concentration generally decreases as transmembrane equilibrium is gradually lost. Calcium concentration may also exhibit postmortem alterations due to cellular disintegration and redistribution processes. These biochemical changes offer potential markers for estimating PMI in a more objective manner. Despite growing evidence supporting biochemical approaches to PMI estimation, comparative studies simultaneously evaluating electrolyte changes in synovial fluid and cerebrospinal fluid remain limited, particularly within the Indian forensic context. Therefore, the present study aims to analyze postmortem changes in Na^+, K^+, and Ca^{2+} concentrations in synovial fluid and cerebrospinal fluid and assess their correlation with PMI. By comparatively evaluating these compartmental fluids, the study seeks to contribute toward developing a more objective, reliable, and reproducible framework for time since death estimation.

Aim:

This study aims to correlate the changes in electrolyte level (Na+, k+, Ca+2) in CSF & Synovial Fluid with postmortem interval.

Objectives:

1. Estimate the levels of electrolytes in CSF & SF.
2. To determine the correlation between electrolyte levels in CSF and SF with different Postmortem Intervals.
3. To obtain the regression formulae to estimate TSD from electrolytes with different.

Materials and Methods

Methodology

This cross-sectional comparative study was conducted in the Department of Forensic Medicine and Toxicology with the collaboration of Department of Biochemistry, King George's Medical University, Lucknow, India, with a total sample size of 100 cases. All deceased bodies brought for medicolegal autopsy within 24 hours of death and having a known and documented time since death (TSD) were considered for inclusion. To improve accuracy in determination of time since death, only hospital deaths certified by a registered medical practitioner were included. Cases were categorized into four groups according to the postmortem interval:

- Group I: 0–6 hours
- Group II: 7–12 hours
- Group III: 13–18 hours
- Group IV: 19–24 hours

The interval between death and sample collection corresponded to the designated study group, and all biological samples were collected during autopsy immediately after opening the relevant anatomical compartments to minimize post-collection biochemical alterations. Cases with pre-existing conditions likely to affect electrolyte concentrations were excluded. Exclusion criteria included cases with known joint diseases, metabolic disorders, meningitis, encephalitis, electrolyte imbalance disorders, chronic renal disease, and deaths resulting from thermal, electrical, or chemical injuries. Additionally, railway track accidents involving extensive trauma to the head or spine, decomposed bodies, mutilated bodies, crushed bodies, and cases showing significant contamination of body fluids were excluded from the study.

Environmental Conditions During Autopsy: Autopsies were performed under standard mortuary conditions at the Department of Forensic Medicine and Toxicology. Ambient temperature and environmental conditions prevailing during autopsy were recorded for each case, as environmental factors may influence postmortem biochemical changes. The mortuary temperature during the study period generally ranged between 21°C to 25°C.

Sample Collection and Handling: Synovial fluid and cerebrospinal fluid samples were collected aseptically during autopsy using sterile disposable syringes and transferred immediately into clean, labeled containers. Samples showing visible contamination with blood, gross particulate matter, putrefactive changes, or inadequate volume were excluded from analysis. Hemolyzed samples were also excluded to minimize analytical interference in electrolyte estimation. Following collection, samples were transported promptly to the laboratory in biochemistry department. Until biochemical analysis, samples were stored at 2–8°C for short-term preservation.

Figure 1 Collection of SF through lateral puncture.

Synovial Fluid: Synovial fluid samples were obtained from the knee joint, preferably from either side, and collected into plain red-top tubes using a 16-gauge needle as shown in Fig;1. The choice of knee, as well as the approach for sample collection, was determined on a case-to-case basis depending on anatomical accessibility and procedural feasibility. The study done by Srettabunjong et al,[6] indicated no significant variation in the biochemical composition of synovial fluid collected from either knee joint, thereby allowing flexibility in sample site selection. With the body in supine position, 2 mL synovial fluid was aspirated from the suprapatellar pouch using an 18-gauge needle. After collection, the sample is promptly sent for analysis in lab.

To separate the cellular components centrifuged the sample at 5000 rpm for 10 minutes, and the supernatant used. Due to high viscosity, SF was diluted (0.05 mL sample with 0.95 mL distilled water), and results were corrected using a dilution factor of 20.

Cerebrospinal Fluid (CSF): CSF was collected by lumbar puncture method of Priya A et al. under aseptic precautions, with the body in sitting or lateral position, was determined on a case-to-case basis depending on anatomical accessibility and procedural feasibility, inserting a spinal needle at the L3–L4 space directed towards the umbilicus. The needle was advanced through ligaments with initial resistance; a brief increase occurred at dura penetration, followed by loss of resistance indicated entry into subarachnoid space. The Stylet was removed and flow of CSF confirmed correct placement. Approximately 5 mL of CSF was collected in a sterile vial without anticoagulant, processed within three hours, centrifuged at 5000 rpm for 5 minutes, and the supernatant was used for analysis.[7]

Biochemical Analysis: Biochemical analysis of both synovial fluid and cerebrospinal fluid samples was performed in the Clinical Biochemistry Laboratory under the Department of Biochemistry. Electrolyte estimation for sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}) was performed using an InnoLyte Plus electrolyte analyzer according to the manufacturer's instructions. Before sample processing, the analyzer was calibrated using standard calibration solutions supplied by the manufacturer.

Quality control procedures included routine internal quality control using commercially available control materials at normal and pathological concentration ranges. Calibration verification and quality control assessments were performed daily before analysis to ensure analytical accuracy and reproducibility. Samples were analyzed in accordance with standard laboratory protocols to minimize pre-analytical and analytical variability.

Ethical Considerations: This research was approved by the Institutional Ethics Committee of King George's Medical University, Lucknow (Approval Letter No. 1733/Ethics/2023, dated 26 September 2023). Informed consent was obtained from guardians, close relatives, or legally acceptable representatives before collecting each sample. All information gathered during the study was completely anonymized, and strict measures were implemented to protect the confidentiality and integrity of the data throughout the research process.

Statistical Analysis: The relationship between electrolyte concentrations and postmortem interval was analyzed using Jamovi 2.3.37 with descriptive and inferential statistics.

Results

Study characteristics: A total of 100 cases were included. The mean age was 31.46 years (SD 10.70), and 72 (72.0%) cases were male. Hanging accounted for 72 (72.0%) deaths and poisoning for 28 (28.0%). The mean time since death (TSD) was 14.14 hours (SD 6.21). The four postmortem-interval groups comprised 18 (18.0%), 23 (23.0%), 28 (28.0%), and 31 (31.0%) cases, respectively [Table 1].

Table 1: Study characteristics (N = 100)

Characteristic	Value, n (%) or mean (SD)	Range
Age in years	31.46 (10.70)	14-73
TSD in hours	14.14 (6.21)	2.17-23.33
Sex		
Male	72 (72.0%)	
Female	28 (28.0%)	
Cause of death		
Hanging	72 (72.0%)	
Poisoning	28 (28.0%)	
Postmortem-interval group		
0-6 hours	18 (18.0%)	
7-12 hours	23 (23.0%)	
13-18 hours	28 (28.0%)	
19-24 hours	31 (31.0%)	

TSD, time since death.

Electrolyte concentrations across postmortem-interval groups: Electrolyte concentrations differed significantly across postmortem-interval groups for CSF sodium, potassium, and calcium and for synovial-fluid potassium and calcium after Holm adjustment; synovial-fluid sodium did not differ significantly [Table 2]. Potassium showed the clearest temporal pattern. Mean CSF potassium increased from 16.21 (SD 4.33) in the 0-6-hour group to approximately 23 in each later group, whereas mean synovial-fluid potassium increased from 6.13 (SD 1.27) to 8.65 (SD 2.00) across the first and last groups. In pairwise comparisons, both CSF and synovial-fluid potassium were lower in the 0-6-hour group than in each later group (all Holm-adjusted $p < 0.001$); no significant differences were observed among the three later groups.

Table 2: Electrolyte concentrations by postmortem-interval group

Analyte	0-6 h (n = 18)	7-12 h (n = 23)	13-18 h (n = 28)	19-24 h (n = 31)	Welch F (df1, df2)	Adjusted p	η^2
CSF sodium	139.93 (8.47)	131.84 (7.88)	132.30 (5.25)	133.68 (8.60)	4.17 (3, 46.95)	0.032	0.129
CSF potassium	16.21 (4.33)	23.81 (2.94)	23.44 (4.78)	23.10 (5.86)	14.22 (3, 49.73)	<0.001	0.264
CSF calcium	2.85 (0.45)	2.50 (0.54)	2.52 (0.53)	2.29 (0.83)	3.53 (3, 51.53)	0.042	0.084

SF sodium	138.62 (7.50)	136.08 (10.39)	139.66 (7.90)	142.84 (10.95)	1.83 (3, 50.24)	0.154	0.068
SF potassium	6.13 (1.27)	7.81 (1.10)	7.93 (1.37)	8.65 (2.00)	11.44 (3, 50.71)	<0.001	0.246
SF calcium	3.03 (0.57)	2.64 (0.84)	3.46 (0.65)	3.18 (0.81)	5.15 (3, 50.48)	0.014	0.145

Values are mean (SD), expressed in mmol/L. Adjusted p values were obtained using the Holm procedure across the six analytes. CSF, cerebrospinal fluid; SF, synovial fluid.

Table 3: Pearson correlations between electrolyte concentrations and exact time since death.

Analyte	r	95% CI	p	Adjusted p
CSF sodium	-0.200	-0.381 to -0.003	0.047	0.093
CSF potassium	0.373	0.191 to 0.531	<0.001	<0.001
CSF calcium	-0.274	-0.447 to -0.082	0.006	0.023
SF sodium	0.187	-0.010 to 0.370	0.063	0.093
SF potassium	0.467	0.298 to 0.608	<0.001	<0.001
SF calcium	0.224	0.029 to 0.402	0.025	0.076

Adjusted p values were obtained using the Holm procedure across the six correlations. CI, confidence interval

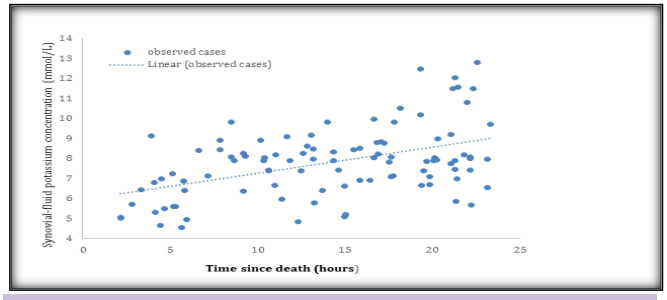

Figure 2. Relationship between synovial-fluid potassium concentration and time since death. Each point represents one case, and the dotted line represents the fitted linear regression line. Synovial-fluid potassium showed a significant positive correlation with time since death (Pearson's r = 0.467, p < 0.001).

Correlation of electrolytes with exact time since death: Synovial-fluid potassium showed the strongest positive correlation with exact TSD, followed by CSF potassium [Table 3; Figure 2]. CSF calcium showed a weak inverse association. After correction for multiple testing, CSF potassium, CSF calcium, and synovial-fluid potassium remained statistically significant.

Regression analysis: The synovial-fluid three-electrolyte model explained more variability in TSD than the corresponding CSF model. The combined six-electrolyte model provided the best fit, explaining 41.4% of the observed variation in TSD (adjusted R^2 = 0.376; [Table 4]). Within the combined model, synovial-fluid potassium and calcium were positive independent predictors, whereas CSF calcium was an inverse predictor [Table 5].

Table 4: Performance of multivariable regression models for estimating time since death

Model	R^2	Adjusted R^2	F (df1, df2)	p	LOOCV RMSE, h	LOOCV MAE, h	PMI-group accuracy
CSF model	0.190	0.164	7.49 (3, 96)	<0.001	5.85	5.05	24%
Synovial-fluid model	0.259	0.236	11.21 (3, 96)	<0.001	5.59	4.58	36%
Combined model	0.414	0.376	10.96 (6, 93)	<0.001	5.19	4.15	43%

The CSF and synovial-fluid models each included sodium, potassium, and calcium from the respective fluid. The combined model included all six electrolyte measurements.

LOOCV, leave-one-out cross-validation; PMI, postmortem interval.

Table 5: Coefficients of the combined six-electrolyte regression model

Term	B	SE	t	p	95% CI for B
Intercept	6.849	11.095	0.62	0.539	-15.183 to 28.881
CSF sodium	-0.092	0.074	-1.25	0.213	-0.239 to 0.054
CSF potassium	0.116	0.111	1.04	0.299	-0.104 to 0.336
CSF calcium	-3.466	0.899	-3.86	<0.001	-5.251 to -1.682
SF sodium	0.057	0.062	0.92	0.360	-0.066 to 0.180
SF potassium	1.263	0.343	3.68	<0.001	0.582 to 1.943
SF calcium	2.570	0.738	3.48	<0.001	1.104 to 4.036

Outcome: exact time since death in hours. CI, confidence interval; CSF, cerebrospinal fluid; SF, synovial fluid.

The resulting equation was: TSD (hours) = 6.849 - 0.092(CSF sodium) + 0.116(CSF potassium) - 3.466(CSF calcium) + 0.057(SF sodium) + 1.263(SF potassium) + 2.570(SF calcium). On leave-one-out cross-validation, the combined model had a root mean square error of 5.19 hours and a mean absolute error of 4.15 hours, with 43% accuracy for classification into the four postmortem-interval groups [Figure 3].

Figure 3: Observed versus leave-one-out cross-validated predicted time since death from the combined six-electrolyte regression model. The dashed solid line represents perfect agreement between observed and predicted values.

Discussion

This comparative autopsy study evaluated postmortem sodium, potassium, and calcium concentrations in cerebrospinal fluid (CSF) and synovial fluid (SF) during the first 24 hours after death. SF potassium showed the strongest correlation with exact time since death (TSD; r=0.467), followed by CSF potassium (r=0.373), whereas CSF calcium declined with increasing TSD (r=−0.274). The SF electrolyte model explained more variation than the CSF model, while the combined six-electrolyte model provided the best fit (R^2=0.414; adjusted R^2=0.376). Nevertheless, cross-validated error remained appreciable, with a mean absolute error of 4.15 hours and only 43% accuracy for classification into the four postmortem-interval groups. These results support electrolyte analysis as an adjunct rather than a standalone method of death-time estimation.

The predominance of potassium as a temporal marker is consistent with early CSF research. Mason et al. demonstrated progressive postmortem increases in CSF potassium and that CSF potassium levels were related to the time elapsed after death, not just to the serum potassium level at the time of death.[8] They also noted a significant interindividual dispersion and overlap in postmortem intervals, suggesting that potassium may offer an approximate temporal indication, but not a precise death time. The moderate CSF potassium correlation and the plateau after 6 hours also indicate a rapid rise in CSF potassium followed by a decrease in temporal discrimination. Yadav et al. then studied CSF sodium and potassium levels in the first 25 postmortem hours.[9] The average hourly change in potassium was 1.21 mEq/L, in sodium was approximately 1.12 mEq/L, and both were significantly related to TSD; the sodium-to-potassium ratio was also a helpful association with the postmortem interval. In our study, CSF potassium rose from 16.21 mmol/L in the 0–6 hour group to about 23 mmol/L in the later groups, although the later groups were not clearly differentiated. The CSF sodium was initially lower with increasing TSD, but became insignificant after multiple-testing correction. The weaker relationship with sodium may be due to differences in case selection, ambient conditions, analytical systems, and group-based versus continuous analysis.

Arroyo et al. measured several CSF biochemical parameters instead of just one electrolyte.[10] They showed that CSF composition alters post mortem, that the temporal stability of the analytes is not equal and that agonal and pathological processes can affect interpretation. The study thus confirmed the value of multianalyte assessment but warned against considering CSF chemistry as independent of cause of death or premortem physiology. Our combined model is based on this multiparametric principle, but the relatively good cross-validated performance shows that more analytes do not necessarily lead to accurate prediction.

Madea et al. first systematically investigated the suitability of SF as an alternative protected compartment in 74 sudden deaths.[11] They determined sodium, potassium, calcium, chloride, urea, creatinine, and glucose in paired SF and vitreous samples. The authors found that most SF analytes were stable enough to give information on the metabolic status prior to death, and that SF was a valuable alternative when vitreous sampling was not possible. They also highlighted the importance of the postmortem stability of analytes and the need to avoid using serum reference ranges for interpretation of SF. Our results build on this by directly comparing the electrolytes in SF and CSF and demonstrate that SF potassium gave the most robust temporal information of all CSF electrolytes.

Tumram et al. compared SF and vitreous humour in post mortem interval of 1.45 to 35.18 hours.[12] Potassium showed a significant increase in both compartments, the most consistent relationship with the death interval, and allowed regression equations to be developed. They used their predictive model, which included potassium as well as age and ambient temperature, recognizing that postmortem biochemical change is a function of both biological and environmental factors. Similarly, SF potassium rose from 6.13 mmol/L at 0–6 hours to 8.65 mmol/L at 19–24 hours and was an independent predictor in the combined model in the present study. The ambient temperature range was relatively small (21–25°C), however, and temperature was not included in the regression equation, which could have reduced the generalisability of the model.

In a subsequent study, Tumram et al.[13] reported a significant post mortem rise in SF potassium and developed a regression-based method to estimate TSD for SF potassium. The relationship was not as consistent during the first 12 hours, the variability increased with the length of the interval, and the authors warned that the one equation should only be used under conditions similar to those under which it was developed. A similar pattern emerges from our data: CSF and SF potassium clearly distinguished the 0–6-hour group from the later groups, but neither reliably distinguished the 7–12-, 13–18-, and 19–24-hour groups. Therefore, potassium could be more useful for differentiating very early from later post mortem intervals than for assigning narrow time bands during the first day.

Siddamsetty et al. studied the SF potassium, sodium, chloride, glucose and calcium in semi-arid climatic conditions.[14] Potassium had the most helpful positive correlation with TSD, while glucose decreased and the relationship of sodium and calcium was less consistent. They emphasized the importance of regional climate and recommended the use of locally derived regression equations instead of universal conversion formulae. The present results are similar in showing a strong positive association between SF potassium and weaker and inconsistent associations between SF sodium and calcium. Interestingly, SF calcium was not significant as a simple correlation but was a significant and positive predictor in the combined model, indicating that its significance may only become apparent after the other electrolytes have been accounted for.

Swain et al. compared CSF and vitreous chemistry in 100 medicolegal autopsies with known death times.[15] There were significant relationships between potassium, glucose and sodium with TSD in both fluids, no meaningful sex-related differences were found for the main biochemical variables and vitreous humour gave more accurate estimates than CSF. Vitreous potassium was the most consistent single marker measured. Vitreous humour was not analysed in our study, but the better performance of SF compared to CSF indicates that SF may be a practical alternative protected compartment in situations where

the eye has been injured, has undergone previous eye surgery, has suffered severe facial injury, or where vitreous samples are unavailable.

Recently, Vieira et al. compiled evidence on the biochemistry of vitreous and SF for early postmortem interval estimation.[16]. Their meta-analytical evaluation concluded that potassium was the most promising SF biomarker, that there was more variation in the results for sodium, calcium, and other constituents, and that there was significant variation in sampling, temperature control, laboratory methods, and regression equations. The review confirmed that SF potassium is a useful supplementary marker but that methodological standardization and external validation is still required. This interpretation is similar to our results, which showed that the univariable model with SF potassium had the highest correlation, and the combined model had a better fit, but still yielded a cross-validated RMSE of 5.19 hours.

The observed postmortem increase in potassium is biologically reasonable. Once the circulation stops, ATP depletion disrupts membrane ion pumps, membranes become nonselective, and intracellular potassium moves into the surrounding extracellular fluids. Exposed to broad neural and meningeal tissue, CSF may experience a rapid early increase and then level off. SF is contained within the joint cavity and may diffuse more slowly from synovium and periarticular tissues, which may account for the more gradual relationship with TSD. The pattern of CSF calcium is inconsistent, and a decrease in CSF calcium may be due to tissue uptake, precipitation, redistribution, or changes in protein binding, making it less useful as a single time marker.

One of the most important findings of this study is the direct, same-case comparison of CSF and SF sodium, potassium and calcium, along with multiple testing and leave-one-out cross validation. The results show that statistical association is not enough to ensure forensic predictive accuracy. The model accounted for 41.4% of the variation in TSD, but over half of the variation was not explained and interval classification was wrong in most instances. The regression equation should therefore be considered as provisional and only valid for similar hospital deaths studied within 24 hours under similar mortuary conditions.

There are a few limitations to be taken into account. The sample was limited to hospital deaths with known times of death, mostly hanging and poisoning, which increased the accuracy of the reference times but decreased the variety of causes of death. Interval groups were not equal and the 0-6 hour group had fewer cases. The model did not include body temperature, refrigeration time, season, agonal period or exact pre-autopsy storage conditions. SF had to be diluted due to its viscosity, which added another source of analytical variation. In addition, there was no independent validation cohort and leave-one-out validation may give overly optimistic results when the model is built and validated in a single centre.

Conclusion

Synovial-fluid potassium showed the strongest association with time since death and performed better than cerebrospinal-fluid potassium during the first 24 hours. Combining electrolytes from both fluids improved predictive performance, but the remaining error limits standalone forensic application. These estimates should therefore be interpreted alongside conventional postmortem findings, with external validation required before routine use.

Limitations of the Study

The present study has certain limitations. It was conducted at a single centre with a relatively small sample size, which may limit the generalizability of the findings. The study included only cases with a postmortem interval of up to 24 hours; therefore, the derived regression equations may not be applicable to advanced stages of decomposition. Furthermore, the regression models were not subjected to internal or external validation and should be considered preliminary until validated in larger, multicentric studies. Additionally, only Na^+, K^+, and Ca^{2+} were evaluated; inclusion of other biochemical markers may further improve the accuracy of postmortem interval estimation.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Chaudhary BL, Veena M and Tirpude BH. Potassium concentration in vitreous humour in relation to time since death. J Forensic Med Toxicol 2007; 24: 26–30.
2. Sheikh NA. Estimation of Postmortem Interval According to Time Course of Potassium Ion Activity in Cadaveric Synovial Fluid. Indian Journal of Forensic Medicine & Toxicology. 2007; 1(2): 45–9
3. Coe JI. Vitreous Potassium as a Measure of the Postmortem Interval: An Historical Review and Critical Evaluation. Forensic Science International. 1989; 42(3): 201–13.
4. Salam HA, Shaat EA, Aziz MHA, et al. Estimation of postmortem interval using thanatochemistry and postmortem changes. Alex J Med.2012;48(4): 335–344.
5. Madea B. Methods for determining time of death . Forensic Sci Med Pathol. 2016;12(4):451–485.
6. Srettabunjong S, Thongphap W, Chittamma A. Comparative and correlation studies of biochemical substances in vitreous humor and synovial fluid. J Forensic Sci. 2019;64(3):778–785.
7. Priya A, Anwar T, Verma AK, Singh M, Rupani R, Kumari S. Optimization of cerebrospinal fluid (CSF) extraction from human cadavers: Advancing forensic pathology and postmortem analysis. MethodsX. 2025 Jul 29;15:103543. doi: 10.1016/j.mex.2025.103543. PMID: 40822547; PMCID: PMC12355545.
8. Mason JK, Klyne W, Lennox B. Potassium levels in the cerebrospinal fluid after death. J Clin Pathol. 1951;4(2):231–233. doi:10.1136/jcp.4.2.231
9. Yadav J, Deshpande A, Arora A, Athawal BK, Dubey BP. Estimation of time since death from CSF electrolyte concentration in Bhopal region of central India. Leg Med (Tokyo). 2007 Nov;9(6):309-13. doi: 10.1016/j.legalmed.2007.05.001.
10. Arroyo A, Rosel P, Marrón T. Cerebrospinal fluid: postmortem biochemical study. J Clin Forensic Med. 2005;12(3):153–156. doi:10.1016/j.jcfm.2004.11.001

11. Madea B, Kreuser C, Banaschak S. Postmortem biochemical examination of synovial fluid—a preliminary study. Forensic Sci Int. 2001;118(1):29–35. doi:10.1016/S0379-0738(00)00372-8
12. Tumram NK, Bardale RV, Dongre AP. Postmortem analysis of synovial fluid and vitreous humour for determination of death interval: a comparative study. Forensic Sci Int. 2011;204(1–3):186–190. doi:10.1016/j.forsciint.2010.06.007
13. Tumram NK, Ambade VN, Dongre AP. Thanatochemistry: study of synovial fluid potassium. Alexandria J Med. 2014;50(4):369–372. doi:10.1016/j.ajme.2014.02.005
14. Siddamsetty AK, Verma SK, Kohli A, Verma A, Puri D, Singh A. Exploring time of death from potassium, sodium, chloride, glucose and calcium analysis of postmortem synovial fluid in a semi-arid climate. J Forensic Leg Med. 2014;28:11–14. doi:10.1016/j.jflm.2014.09.004
15. Swain R, Kumar A, Sahoo J, et al. Estimation of post-mortem interval: a comparison between cerebrospinal fluid and vitreous humour chemistry. J Forensic Leg Med. 2015;36:144–148. doi:10.1016/j.jflm.2015.09.017
16. Vieira RB, Vicentin-Junior CA, Damascena NP, et al. Biochemical analysis of vitreous humor and synovial fluid in the estimation of early postmortem interval: a meta-analytical approach. J Forensic Leg Med. 2024;108:102782. doi:10.1016/j.jflm.2024.102782