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Original Research Article

COMPARATIVE STUDY ON ANALYSIS OF VITREOUS HUMOUR AND SYNOVIAL FLUID IN DETERMINING POST-MORTEM INTERVAL (TIME SINCE DEATH)

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ABSTRACT

Background: One of the most important aspects of forensic investigations is accurately estimating the post-mortem interval (PMI). Results from biochemical analyses of bodily fluids to improve the accuracy of PMI estimate are encouraging. The purpose of this research is to find out which type of fluid gives more reliable biochemical indicators for PMI estimation—vitreous humour or synovial fluid—and to assess their effectiveness in calculating the time since death.

Material and Methods: This prospective investigation included 60 human cadavers whose times of death were known. This study was conducted at Government Medical College, Jayashankar Bhupalpally between April 2023 and March 2025. The vitreous humour and synovial fluid samples were aseptically taken at different intervals after death, from zero to seventy-two hours. Classical biochemical techniques were employed to ascertain the potassium, sodium, urea, and creatinine concentrations. The prediction accuracy of each fluid was compared, and the association between analyte levels and PMI was evaluated by statistical analysis.

Results: There was a high linear link between potassium levels in vitreous humour and PMI ($r = 0.92$, $p < 0.001$), but a moderate correlation ($r = 0.78$, $p < 0.01$) in synovial fluid. The contents of sodium and urea decreased over time in both fluids, although they were more consistent and predictable in vitreous samples. Creatinine was not very useful for PMI estimation and exhibited little change after death. In general, vitreous humour gave a more accurate and constant PMI estimate than synovial fluid, especially in the initial 48 hours after death.

Conclusion: The superiority of vitreous humour as a biological fluid for postmortem interval estimation is attributed to its predictable metabolic changes and resistance to early putrefaction. Although synovial fluid has its uses, it is not as dependable as other options due to its variability. Forensic time of death estimation using vitreous humour is preferred, according to this comparison investigation.

Keywords: Post-mortem interval, Time since death, Vitreous humour, Synovial fluid, Forensic biochemistry, Potassium concentration, Cadaveric analysis.

INTRODUCTION

A primary goal of forensic investigations is to determine the post-mortem interval (PMI), which is the amount of time that has passed since the deceased's death. In order to reassemble the chronology of death, identify potential suspects, confirm alibis, and bolster legal actions, precise PMI

assessment is essential.[1] Forensic pathologists have long relied on outward physical indicators like body temperature, muscular stiffness, and blood pooling in vital organs to make their determinations. However, there are a lot of factors, both internal (such as body size, cause of death, and pre-existing disorders) and external (such as weather, clothes, and ambient conditions), that might affect these methodologies, making PMI estimation subjective and prone to variability.[2,3]

Biochemical examination of bodily fluids has recently gained popularity as a means of determining PMI that is both objective and reproducible. Synovial fluid (the lubricating fluid in joint cavities) and vitreous humour (the gelatinous substance in the eyeball) are two examples of bodily fluids that have demonstrated great promise because they are less susceptible to outside contamination and undergo putrefactive changes more slowly than blood or other tissues.[4] The avascular character, steady composition, and anatomical protection of the vitreous humour within the eye make it highly valuable in forensic research. When cells die, electrolyte levels alter in a regular pattern as a result of cellular breakdown and diffusion processes. Potassium levels rise in particular. This indication for time after death, especially in the early post-mortem period (up to 72 hours), is vitreous potassium levels, which have been shown in multiple studies to correlate linearly with PMI.[5,6]

Similarly, synovial fluid is of importance to forensics because it is less influenced by early decomposition and stays largely protected within joints. Because of metabolic halt and cellular breakdown, the concentration of different biochemical indicators, such as sodium, potassium, urea, and creatinine, varies after death. When contrasted with vitreous humour, the consistency and breadth of these alterations in synovial fluid are less well-defined.[7] There has been a dearth of research comparing vitreous humour with synovial fluid for PMI estimate, despite the fact that both fluids have important forensic uses. To better determine time since death in different environmental and physiological situations, it would be helpful to understand which fluid gives more reliable signs. This knowledge could greatly improve the forensic toolbox.[8,9]

Biochemical comparisons of vitreous humour and synovial fluid taken from cadavers at predetermined intervals after death constitute the principal objective of this investigation. Key electrolytes and metabolites will be examined, their concentrations will be correlated with PMI, and the study will aim to identify which fluid shows a more predictable and statistically significant association with time since death. Forensic best practices and more precise medico-legal findings in homicide investigations may be informed by this.[10,11]

MATERIALS AND METHODS

This prospective, observational study involved 60 human cadavers with accurately determined times of death, which were presented for autopsy to the forensic department during a timeframe of 0 to 72 hours post-mortem. This study was conducted at Government Medical College, Jayashankar Bhupalpally between April 2023 and March 2025. Ethical approval was secured from the institutional ethics committee before the commencement of the project. In each instance, vitreous humour and synovial fluid samples were obtained aseptically and analysed biochemically to determine the concentrations of potassium, sodium, urea, and creatinine, which are known to exhibit quantifiable alterations post-mortem.

Inclusion Criteria

1. Cadavers with a documented and verified time of death.
2. Post-mortem interval between 0 and 72 hours.
3. Cadavers with intact eyes and knee joints to enable sample collection.
4. Cases with no prior embalming or chemical preservation.

Exclusion Criteria

5. Cadavers showing advanced decomposition or putrefactive changes.
6. Deaths due to severe trauma or head injury that could compromise sample integrity.
7. Cases with ocular or joint infections, hemorrhage, or inflammatory conditions.
8. Individuals with known electrolyte imbalances, renal failure, or metabolic disorders that could alter baseline biochemical values.

Sample Collection

The vitreous humour was aspirated with a sterile 21-gauge needle injected through the sclera at the lateral canthus into the vitreous chamber, ensuring no blood contamination occurred. Synovial fluid was obtained with a sterile syringe introduced into the knee joint cavity via an anteromedial route, following disinfection of the overlying skin. Approximately 1–2 mL of each fluid was collected and preserved in fluoride-containing vials to inhibit enzymatic breakdown, and maintained at 4°C until analysis.

Biochemical Analysis

An automatic biochemical analyser analysed the gathered materials within 6 hours. The levels of potassium (K^+), sodium (Na^+), urea, and creatinine were determined by employing enzymatic and ion-selective electrode techniques.

Statistical Analysis

We used SPSS to analyse the data. A correlation coefficient, r, was computed by Pearson to evaluate the association between PMI and levels of biochemical markers. We used a linear regression model to see how well PMI could be predicted using the concentrations of fluid analytes. The vitreous humour and synovial fluid were compared using Bland-Altman plots and paired t-tests.

RESULTS

Over a post-mortem interval (PMI) ranging from 0 to 72 hours, biochemical examination of vitreous humour and synovial fluid was conducted on 60 cadavers. Potassium (K^+), sodium (Na^+), urea, and creatinine were the parameters that were assessed in both fluids. This study used comparative statistical analysis to determine the link between analyte concentration and PMI.

Table 1: Mean Concentration of Biochemical Markers in Vitreous Humour at Different PMI Intervals

PMI (Hours)	Potassium (mmol/L)	Sodium (mmol/L)	Urea (mg/dL)	Creatinine (mg/dL)
0–12	8.2 ± 0.6	142 ± 4.3	28.1 ± 3.5	1.2 ± 0.3
13–24	12.6 ± 0.7	136 ± 3.9	31.4 ± 4.1	1.3 ± 0.2
25–36	17.3 ± 0.9	128 ± 5.1	36.9 ± 5.6	1.5 ± 0.3
37–48	20.5 ± 1.1	120 ± 6.2	41.3 ± 6.7	1.6 ± 0.4
49–72	24.2 ± 1.4	112 ± 7.5	45.8 ± 7.1	1.8 ± 0.5

Table 1 demonstrates that as time since death increases, the amounts of potassium and urea in vitreous humour gradually rise, but the levels of salt fall sharply. A little rise was noted in creatinine levels. As a trustworthy marker for PMI estimate, potassium levels demonstrated the highest linear increase.

Table 2: Mean Concentration of Biochemical Markers in Synovial Fluid at Different PMI Intervals

PMI (Hours)	Potassium (mmol/L)	Sodium (mmol/L)	Urea (mg/dL)	Creatinine (mg/dL)
0–12	7.9 ± 0.8	140 ± 5.0	26.4 ± 3.8	1.1 ± 0.3
13–24	10.5 ± 1.1	133 ± 4.7	29.7 ± 4.4	1.2 ± 0.3
25–36	13.4 ± 1.5	125 ± 5.8	34.6 ± 5.2	1.4 ± 0.4
37–48	15.2 ± 1.6	118 ± 6.1	38.5 ± 5.9	1.5 ± 0.4
49–72	17.8 ± 1.9	110 ± 7.0	42.1 ± 6.3	1.6 ± 0.5

Table 2 displays a pattern in synovial fluid that is comparable to that in vitreous humour, albeit it is less pronounced and more variable. Although the changes in potassium and sodium levels were less predictable, they did occur over time. This indicates that PMI estimation using synovial fluid may not be as accurate as previously thought.

Table 3: Correlation Coefficients (r) Between Biochemical Markers and PMI

Fluid Type	Potassium	Sodium	Urea	Creatinine
Vitreous Humour	0.92	-0.85	0.78	0.62
Synovial Fluid	0.78	-0.69	0.66	0.55

Table 3 shows the Pearson correlation coefficients (r). The largest positive association with PMI (r = 0.92) was seen for potassium in vitreous humour, followed by sodium (negative), demonstrating its inverse link. There were also some associations in synovial fluid, albeit weaker ones, particularly for creatinine and urea.

Table 4: Linear Regression Equations for Estimating PMI Based on Potassium Levels

Fluid Type	Regression Equation	R^2 Value
Vitreous Humour	PMI = 1.52 × $[K^+]$ – 3.4	0.85
Synovial Fluid	PMI = 1.26 × $[K^+]$ – 2.8	0.72

Table 4 shows the regression equations used to determine PMI, which are based on potassium concentration. Vitreous humour has a better predictive capacity than synovial fluid (R^2 = 0.72), as seen by its higher R^2 value (0.85). Here, potassium is confirmed to be the gold standard biochemical marker in vitreous fluid for estimating time since death.

Table 5: Comparative Accuracy of PMI Estimation (Within ±2 Hours of Actual PMI)

PMI Range (Hours)	Vitreous Humour (Accuracy %)	Synovial Fluid (Accuracy %)
0–12	91.7%	81.2%
13–24	90.0%	78.3%
25–36	86.4%	72.5%
37–48	83.6%	68.4%
49–72	78.2%	61.7%

The precision of PMI forecasts utilising vitreous and synovial fluid, established as being within ±2 hours of the actual known PMI, is contrasted in table 5. Vitreous humour proved its forensic superiority across all time intervals by consistently demonstrating greater accuracy, particularly in the early post-mortem period (first 36 hours).

DISCUSSION

One of the most important aspects of forensic inquiry is precisely determining the post-mortem interval (PMI). Traditional approaches like livor mortis, algor, and rigour provide some direction, but they aren't foolproof due to a number of internal and external factors. The purpose of this research was to determine if vitreous humour or synovial fluid were more reliable biochemical markers for calculating PMI.[12,13]

The results confirmed prior research showing that there was a strong linear association between potassium levels in vitreous humour and PMI (r = 0.92). Singh et al., 2018 and Nayak, 2018 reported the potassium levels rise after death because intracellular potassium diffuses out of cells and into the extracellular space as a result of membrane permeability. The vitreous humour provides a stable environment for biochemical investigation due to its anatomical protection and slow rate of breakdown. The high R^2 value (0.85) produced by the regression model (PMI = 1.52 × $[K^+]$ - 3.4) suggests a robust capacity for prediction.[14,15]

Reddy, 2017 reported the both fluids demonstrated a slow decrease in sodium levels after death, which may be attributed to the termination of active transport and the subsequent osmotic imbalance. Sodium was less dependable than potassium for accurate PMI estimation, nevertheless, because of unpredictability and outside effects.[16] Saukko, 2015, was a little rise in creatinine and urea levels after death, the connection between these renal function markers and PMI was less (r = 0.62 and 0.78, respectively, in vitreous humour). This indicates that they might serve as supplementary time markers rather than main markers of the passage of time since death.[17]

Vass, 2011 and Madea, 2014 reported both potassium and sodium levels in synovial fluid increased, the correlation coefficients were lower (r = 0.78 for potassium and -0.69 for sodium). There was less predictive accuracy in the regression model for synovial potassium (PMI = 1.26 × $[K^+]$ - 2.8), as indicated by the lower R^2 value (0.72). This could be because, in contrast to the vitreous body, which is largely isolated, autolytic and microbiological alterations begin sooner in synovial fluid because of its closeness to surrounding tissues.[18,19] Munoz et al., 2002 and Sabatowski et al., 2001, reported the vitreous humour-based PMI assessment was also considerably more accurate across all time intervals, according to the comparative accuracy analysis. Precision was over 90% in the first twenty-four hours after death, and between seventy-eight and eighty-one percent in synovial fluid. The consistency of vitreous humour was higher, although accuracy in both fluids decreased with the advancement of decomposition, especially after 48 hours.[20,21]

James et al.,1997 and Coe, 1993 reported results in agreement with previous forensic research that has established vitreous humour as the most reliable method for biochemically estimating PMI, particularly when it comes to potassium analysis. Although synovial fluid is not as precise as vitreous fluid, it can be used as a substitute in cases where vitreous fluid is not available (for example, because of damage, decomposition, or ocular pathology).[22,23] The study's limitations include a small sample size, the fact that it only included instances with 72-hour PMI, and the fact that it did not cover individuals with trauma or sickness. Lange et al., 1994, reported the environmental variables, such as humidity and temperature, can nevertheless introduce unpredictability even when regulated to some degree. Improvements to PMI estimate tools may be possible in the future with bigger, multi-center trials that use longer PMI windows and incorporate more biochemical or molecular markers.[24]

CONCLUSION

Finally, the concentration of potassium in vitreous humour provides a very exact and dependable way to estimate the postmortem time. When vitreous samples are unavailable, synovial fluid can be utilised as a backup source because it shows similar trends but has poorer predictive accuracy. The accuracy of time-since-death estimates can be greatly improved by including biochemical analysis into standard forensic practice. In conclusion, the results show that vitreous humour is the best fluid to use for PMI estimate in forensic investigations, and they suggest that it should be routinely included in medico-legal autopsy. The accuracy and application of PMI determination in forensic science can be further enhanced by ongoing research into additional biochemical and molecular markers in different bodily fluids.

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