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Effects of Cold-Storage Temperature on the Accuracy of Death Interval Estimation Using the Thanatochemical Dynamics of Vitreous Humour and Synovial Fluid

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Research Article

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Abstract

Background: Accurate death interval (DI) estimation is crucial in forensic investigations. Various methods, including temperature-based techniques, postmortem changes, and circumstantial evidence, have been employed for this purpose. Thanatochemistry, which focuses on the biochemical changes after death, offers a promising approach. Body fluids such as vitreous humour and synovial fluid have been extensively studied for their potential in PMI estimation. The use of biochemical markers from body fluids such as vitreous humour and synovial fluid has been widely studied, but the effects of cold storage on these estimates remain a challenge.

Objective: This study aimed to evaluate the impact of cold storage on PMI estimation using regression equations based on potassium, sodium, chloride, and glucose levels in vitreous humor and synovial fluid.

Methods: A 2-year prospective analysis of 170 hospital autopsy cases with known death time was conducted on those bodies exposed to cold storage temperature (4-7oC) before the autopsy. Biochemical analyses were conducted on vitreous humor and synovial fluid samples from bodies subjected to cold storage. Electrolyte/chemical levels (sodium, potassium, chloride, and glucose) were measured in both fluids, and their correlation with death interval was assessed. Regression equations were derived to estimate DI, providing reliable estimates within specific time frames. Regression equations were applied to estimate the death interval (DI) at zero hours of actual death time. The correlations between these chemical markers and PMI were analysed, focusing on the influence of cold storage.

Results: Potassium levels in both vitreous humour and synovial fluid showed a positive correlation with PMI, leading to an overestimation of DI by 1.2 to 1.5 hours. Sodium and chloride levels demonstrated a negative correlation, resulting in

underestimations ranging from 5 to 31 minutes. Glucose levels showed minimal and statistically insignificant variations, indicating limited utility as a PMI marker in cold-stored bodies.

Conclusion: The study findings contribute to understanding the reliability of vitreous humour and synovial fluid analysis in estimating PMI, even in cases where bodies have undergone cold storage. Cold storage significantly affects the accuracy of PMI estimation using biochemical markers. Potassium remains a valuable indicator but tends to overestimate PMI under cold conditions. Sodium and chloride provide supplementary information but are prone to underestimation, while glucose is less reliable. A multi-marker approach, considering the specific environmental conditions, is recommended for more accurate PMI estimation. Further research is needed to refine the regression models for cold storage effects.

Keywords: Thanatochemistry; Vitreous Humour; Synovial Fluid; Death Interval; Estimation; Cold Storage; Temperature

Abbreviations

DI: Death Interval; PMI: Postmortem Interval; VH: Vitreous Humour; SF: Synovial Fluid; ISE: Ion-Selective Electrode.

Introduction

Accurate estimation of death interval (DI) is a longstanding challenge for death investigators. With various currently available methods, it is possible to provide a reasonably close range for DI. However, forensic pathologists often find it challenging to determine the exact time frames or even hours for DI, and when they do, the estimate should be viewed with caution. Pathologists typically rely on temperature-based methods, postmortem changes, changes in the eye, and circumstantial evidence to estimate DI. The significance of postmortem biochemistry has been well researched, and its implications regarding DI have long been recognized.

Forensic pathologists have explored thanatochemical methods to determine the postmortem interval (PMI) using various biochemical markers. The principle of postmortem thanatochemistry lies in electrolyte changes in body fluids and using the rate of electrolyte changes in estimating DI [1]. Body fluids subjected to examination are blood, serum, CSF, vitreous humour (VH), and synovial fluid (SF) [2].

Among these, the analysis of vitreous humour has been extensively studied due to its relatively isolated nature and resistance to early postmortem changes [1-15].

Numerous studies have been carried out with reference to vitreous humour biochemistry and have justified its importance in assessing DI [13,16-29]. Potassium concentration in the vitreous humor, in particular, has shown promise as a reliable indicator of PMI, with several studies providing substantial evidence to support this correlation [16-29]. Additionally, synovial fluid has recently gained attention as a potential marker, especially in cases involving joint injuries [10-11]. Currently, postmortem electrolyte changes in synovial fluid have been widely researched regarding DI, and investigators have substantiated their usefulness on par with vitreous humor in estimating DI.

While previous studies have explored the use of vitreous humor and synovial fluid in PMI estimation, the influence of cold storage temperature on the accuracy of these methods remains unclear. Many forensic cases involve bodies kept in cold storage, either at the scene of death or during transportation to the morgue. This study aimed to investigate the effects of cold storage temperature on the thanatochemical dynamics of vitreous humor and synovial fluid, and their subsequent utility in estimating PMI [29-32].

Moreover, existing studies suffered from several limitations, including a small sample size and the use of older chemical analysis techniques, such as flame photometry [11,29]. In these studies, sample collection methods were independent of regional climatic temperature and cold storage preserved bodies. Therefore, given the importance of DI and the scientific utility of vitreous humour and synovial fluid, this study aimed to estimate the death interval from biochemical analysis of vitreous humour and synovial fluid using advanced technology for biochemical analysis of these fluids and assessing the effects of cold storage on the death interval (DI).

Materials and Methods

Study Design

This two-year prospective study involved 170 autopsy cases from the Department of Forensic Medicine and Toxicology, JIPMER, a National Medical Research Institute in South India. The study included hospital deaths that were medically certified, with documented dates and times of death. A study was conducted on 170 autopsy cases stored at

temperatures ranging from +4°C to +7°C in cold chambers for varying durations after death and before sample collection and analysis.

Patients with pre-existing metabolic disorders, dehydration, ocular diseases, ocular trauma, or those who were declared dead before being admitted to the hospital were excluded. Individuals with knee joint injuries or cellulitis of the lower limbs were excluded from the study.

Sample Collection and Analysis

Using a 20-gauge hypodermic needle without applying excessive pressure, the tip was inserted lateral to the limbus, and the non-contaminated vitreous humor (1.5 mL) was aspirated from the outer palpebral fissure of the eye. Sterile water was injected into the eyeball for cosmetic restorations. A 10 ml syringe with an 18-gauge needle was used to puncture the suprapatellar pouch from the lateral side while the patient was in a supine position. Synovial fluid crystallizes during aspiration because the body is kept in a cold chamber. Therefore, the body was thawed for 30 min before aspirating synovial fluid using tap water over the knee joint. Samples were sent for analysis immediately after collection. Before analysis, synovial fluid was diluted with distilled sterile water in a 1:4 dilution to prevent clogging of the aspirator component of the analyzer, and the vitreous humor sample was analyzed directly without dilution. Beckman Coulter Analyzer AU680 was used to analyse samples for electrolytes and glucose. The Beckman Coulter AU680 System employs an ion-selective electrode (ISE) module for Na+, K+, and Cl- and crown ether membrane electrodes for sodium-, potassium-, and molecular-oriented PVC membranes for chloride. The Beckman Coulter AU680 System uses the hexokinase G-6-PDH method to measure glucose levels. After analyzing the samples, the value of the synovial fluid was multiplied by 4 to account for the dilution factor before entering the data.

Statistical analysis Categorical data, such as sex, cause of death, manner of death, and autopsy findings, were expressed as frequency and percentage. Continuous data, including age, biochemical parameters, and DI, are expressed as mean ± SD or median ± range. The relationship between the biochemical parameters of synovial fluid and vitreous humor and DI was determined using correlation analysis. Regression analysis was carried out to establish a relationship between the concentrations of biochemical parameters (sodium, potassium, chloride, and glucose) and DI. Statistical analysis was performed at a 5% significance level, with a p-value of < 0.05. was considered statistically significant. Statistical analyses were performed using Excel 365 (Microsoft) and SPSS Software version 18. Higher-order statistics were analyzed using multivariate regression analysis. The effects of cold storage temperature on the accuracy of the estimated DI were calculated by comparing known and estimated death intervals.

Ethics Approval

This study was conducted in the morgue attached to the Department of Forensic Medicine and Toxicology in collaboration with the Department of Biochemistry at Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India. Ethical approval was obtained from the Institutional Ethics Committee.

Results

Post-Mortem Interval and Biochemical Dynamics: The postmortem interval ranged from 4 to 93 h, with a mean of 20.19 hours and standard deviation of 26.35. The samples were categorized into four DI ranges: 0-12 hours, 12-24 hours, 24-36 hours, and >36 h. The time-dependent postmortem changes in the vitreous humor and synovial fluid chemical levels after death are shown in Table 1.

Death Interval (hr)	Vitreous Sodium (mmol/L)	Synovial Sodium (mmol/L)	Vitreous Potassium (mmol/L)	Synovial Potassium (mmol/L)	Vitreous Chloride (mmol/L)	Synovial Chloride (mmol/L)	Vitreous Glucose (mmol/L)	Synovial Glucose (mmol/L)
0-12	58-228	26-161	3.39-19	1-6.8	30.0-194	21-124	0-310	0-140
24-Dec	86-226	24-140	1-15.6	0.8-12	12-190	20-148	0-310	0-140
24-36	103-262	28-162	2.92-16	1-8.6	45-200	26-88	0-310	0-140
>36	44-186	30-162	1-13.4	1-13.2	17-174	26-74	0-310	0-140

Table 1: Descriptive Statistics of Postmortem Changes in Vitreous Humor and Synovial Fluid Chemicals Over Time (N=170).

Descriptive statistics and concentrations of biochemical constituents in the vitreous humor and synovial fluid across these intervals are presented in Table 2. The sodium concentration in the vitreous humor ranged from 44 to 262 mmol/L (p <0.001) with an SD (29.527). The sodium concentration in the synovial fluid ranged from 24 to 162 mmol/L (p <0.001), and the SD was 27.7816. The potassium concentration in the vitreous humor ranged from 1 to 19.2 mmol/L, p <0.001) and SD (2.995). Potassium concentration in synovial ranged from 0.8 to 13.2 mmol/L, p value <0.001,

and SD of 2.2713. The chloride concentration in the vitreous humor ranged from 17 to 200 mmol/L (p <0.001), and the SD was 28.54. The chloride concentration in the synovial fluid ranged from 20 to 148 mmol/L (p <0.001), and SD was 20.8185. We observed a positive correlation between potassium, sodium, and chloride concentrations in the vitreous humor and synovial fluid over time since death. Because a positive correlation was obtained, we were able to derive a regression equation.

- DI = 9.093 × (vitreous potassium) + 1.247 (r2 = 0.3)
- DI = 14.498 × (synovial potassium) + 1.542 (r2 = 0.3)
- DI = 23.624 x (vitreous sodium) - 0.024 (r2 = 0.003)
- DI = 20.803 x (vitreous chloride) - 0.005 (r2=0.012)
- DI = 22.192 x (synovial chloride) - 0.031 (r2=0.051)

The glucose concentration in the vitreous humor ranged from 0 to 310 mmol/L (p >0.05) and the SD (53.23). The glucose concentration in the synovial fluid ranged from 0 to 140 mmol/L (p >0.05) and the SD (26.25). A negative correlation was observed between glucose concentration in the vitreous humor and synovial fluid with time since death.

- DI = 20.169 x (vitreous glucose) + 0.001 (r2=0.004)
- DI = 20.975 x (synovial glucose) - 0.03 (r2=0.062)

The relationship between all body fluid chemicals and the studied ranges of death intervals was found to be statistically significant, but the strength of this correlation varied. Glucose was the only exception, as it did not show a significant correlation with postmortem interval (Tables 1 & 2). Variable degrees of positive correlations were found between sodium, potassium, and chloride concentrations in both fluids and DI, enabling the derivation of regression equations. In contrast, negative correlations were observed between glucose concentrations in both fluids and the DI.

Vitreous humour and Synovial fluid electrolyte concentration		0-12 Hrs		12-24 Hrs		24-36 Hrs		> 36 Hrs	
		VH	SF	VH	SF	VH	SF	VH	SF
Sodium	Range	58-228	26 -161	86-226	24-140	103-262	28-162	44-168	36-124
	Mean	139.5	78.02	146.3	83.85	142.95	82.14	134.3	92.4
	SD	30.3	30.7	27.46	26.62	33.29	28.312	36.145	27.81
	95 % CI	±9.58	±9.51	±5.85	±5.56	±14.19	±11.83	±22.86	±17.586
	p value	p < 0.001 (0.000)		p < 0.001 (0.000)		p < 0.001 (0.000)		p < 0.05 (0.037)	
Potassium	Range	3.39-19	1-6.8	1- 15.6	0.8-12	2.92-16	1-8.6	5.4-19.2	2.3-13.2
	Mean	8.089	2.91	8.949	3.81	8.84	3.35	11.91	6.57
	SD	2.78	1.36	2.674	2.187	3.296	1.988	4.186	3.886
	95 % CI	±8.79	±0.42	±0.57	±0.46	±1.41	±0.83	±2.648	±2.458
	p value	p < 0.05 (0.029)		p < 0.001 (0.000)		p < 0.001 (0.000)		p < 0.05 (0.021)	
Chloride	Range	30.0-194	21-124	12-190	20-148	45-200	26-88	13-147	40-92
	Mean	114.1	62.8	118.6	65.3	113.89	58.7	105.7	64.5
	SD	30.05	22.014	30.2	21.72	32.856	14.416	36.724	20.501
	95 % CI	±9.31	±6.82	±6.31	±4.54	±13.73	±6.02	±22.76	±12.97
	p value	p < 0.001 (0.000)		p < 0.001 (0.000)		p < 0.001 (0.000)		p < 0.05 (0.009)	
Glucose	Range	0-200	0-140	0-310	0-128	0-288	0-52	1-120	Apr-92
	Mean	38.37	29.4	23.84	25.9	32.91	16.64	34.4	34.2
	SD	54.23	30.47	45.85	25.38	80.42	14.31	47.23	33.31
	95 % CI	±16.82	±9.44	±9.58	±5.3	±33.6	v5.98	±29.27	±20.65
	p value	p > 0.05 (0.280)		p > 0.05 (0.648)		p > 0.05 (0.356)		p > 0.05 (0.989)	
*p<0.05. Glucose levels are expressed in terms of median with minimum and maximum values in parenthesis; other chemicals are expressed in terms of mean with SD. VH – vitreous humour; SF – synovial fluid									
All calculations were done at 95% confidence interval (CI) with a significance threshold of 0.05 (p < 0.05).									

Table 2: Show Descriptive Statistics of Time-Dependent Postmortem Changes in Vitreous Humour and Synovial Fluid Chemicals Over Time After Death (N=170).

To explore the higher-order relationship, we derived the following least square regression equations for the postmortem concentration changes of sodium, potassium, chloride, and glucose in synovial fluid and vitreous humor:

- Y= 11.612+ (synovial sodium) x 0.042+ (vitreous sodium) x 0.055

The least square regression equation for Potassium in synovial fluid and vitreous humour is

- Y= 15.09+ (synovial potassium) x 0.781- (vitreous potassium) x 0.007
- The least square regression equation for Chloride in synovial fluid and vitreous humour is

- Y= 28.35- (synovial chloride) x 0.002- (vitreous chloride) x 0.095
- The least square regression equation for glucose in synovial fluid and vitreous humour is

- Y= 18.32- (synovial glucose) x 0.014+ (vitreous glucose) x 0.16

The least square regression equations for all chemicals showed significant but variable degrees of improvisation in the death interval estimates.

The effects of cold storage on the DI estimates of both body fluids (vitreous humor and synovial fluid) are presented in Table 3. All chemicals, except glucose, demonstrated statistically significant DI estimates with some degree of variability in the estimates. Potassium levels in both fluids were found to have a positive correlation with PMI, resulting in an overestimation of the death time by 1.5 to 1.2 hours. In contrast, chemicals with negative correlations underestimated DI, ranging from 31 min to 5 min (Table 3).

Regression equations & coefficient of determinants	Estimated death interval at zero hours of actual death time.	P value
DI = 9.093 × (vitreous potassium) + 1.247 (r2 = 0.3)	Overestimated 1.2 hours	0.001
DI = 14.498 × (synovial potassium) + 1.542 (r2 = 0.3)	Overestimated 1.5 hours	0.001
DI = 23.624 x (vitreous sodium) - 0.024 (r2 = 0.003)	Underestimated by 24 mins	0.001
DI = 20.803 x (vitreous chloride) - 0.005 (r2=0.012)	Underestimated by 5 mins	0.001
DI = 22.192 x (synovial chloride) - 0.031 (r2=0.051)	Underestimated by 31 mins	0.001
DI = 20.169 x (vitreous glucose) + 0.001 (r2=0.004)	Overestimated 10 mins	0.99
DI = 20.975 x (synovial glucose) - 0.03 (r2=0.062)	Underestimated by 30 mins	0.989

Table 3: Illustrate the Death Interval Estimates Using Vitreous Humor and Synovial Fluid Chemical Regression Equations in Bodies Kept in Cold Storage Before Death.

Discussion

The results of this study reinforce the reliability of vitreous humour as a valuable matrix for estimating the postmortem interval (PMI), particularly through the measurement of potassium levels. Numerous studies Rodriguez DR, et al. [1-12], have shown that vitreous potassium levels increase in a predictable linear pattern after death. This linearity is critical, as it allows forensic pathologists to estimate the PMI with greater accuracy, even under varying environmental conditions. Additionally, the resistance of vitreous humour to early postmortem changes, as discussed by Coe JI [4], further enhances its utility as a forensic tool.

Beyond potassium, other biochemical markers in the vitreous humor, such as glucose and sodium, have been explored for PMI estimation, with mixed results. Studies by Madea B, et al. [33] have highlighted the challenges of using these markers, particularly due to their variability and susceptibility to environmental factors. Despite these challenges, these markers may still provide supplementary information in certain forensic contexts, particularly when used alongside potassium. The inclusion of these additional markers could potentially refine DI estimates, particularly in cases in which potassium levels alone might not provide sufficient resolution.

The comparative picture of the postmortem thanatochemical dynamics of both vitreous humour and synovial fluid is discussed below in light of existing research (Tables 2 & 3).

Potassium Dynamics

Potassium levels in both vitreous humour and synovial fluid showed a strong positive correlation with DI, particularly within the first 40 hours postmortem. This correlation aligns with previous studies, although the precision in this study was enhanced owing to the use of advanced analytical techniques. The regression equations derived from this study can be reliably used to estimate DI up to 80 h postmortem, although with a lower r2 value than previous studies.

Sodium and Chloride Dynamics

Changes in sodium concentration in the vitreous humor correlated positively with DI for up to 36 h, after which the correlation diminished. Synovial fluid sodium levels were not significantly correlated with DI. The chloride levels in both fluids decreased over time, with a strong positive correlation up to 40 h postmortem. Regression equations for chloride can be used effectively within this timeframe.

Glucose Dynamics

Glucose concentration in both the vitreous humor and synovial fluid demonstrated a negative correlation with DI, particularly within the first 40 h postmortem. This finding is consistent with those of previous studies and suggests that glucose levels can be a useful indicator of DI in cases where other fluids are unavailable.

This study also explored the potential of synovial fluid as an alternative or complementary matrix for PMI estimation. Although synovial fluid has not been as extensively studied as vitreous humor, many studies Singh G, et al. [8-19] suggests that it could offer valuable insights, particularly in cases involving joint trauma. The relative isolation of synovial fluid from early postmortem changes, similar to the vitreous humour, could make it a useful alternative, especially in cases where ocular tissues are compromised. However, further research is necessary to establish standardized protocols for forensic investigation.

Overall, the findings of this study align with those of previous research, which established potassium concentration in the vitreous humor as a reliable marker for estimating PMI [11-14]. Madea B, et al. [9] demonstrated that vitreous potassium levels increased linearly after death, providing a consistent marker for PMI estimation. This was corroborated by Srettabunjong S, et al. [12], who also highlighted the robustness of this method under various environmental conditions. Moreover, the potential of other biochemical markers, such as glucose and sodium levels in the vitreous humor, has been explored, although with varying degrees of reliability [10-15,18-25]. In this context, some studies by Poovaragavan V, et al. and Madea B, et al. [33,34] are particularly noteworthy, as they discuss the broader applicability of ocular and synovial fluid analysis in forensic pathology.

The present study integrated multiple biomarkers and observed enhanced accuracy in death interval estimation. Many studies observed similar observations by integrating multiple markers for DI estimations [24-33].

Influence Cold-storage Temperature of DI Estimates

The findings of this study provide important insights into the impact of cold storage on postmortem interval estimation using vitreous humour and synovial fluid chemistry. Potassium levels in both fluids demonstrated a strong positive correlation with PMI, leading to an overestimation of the death interval (DI) by 1.2 hours in the vitreous humour and 1.5 hours in the synovial fluid. This overestimation aligns with previous research, suggesting that cold storage may slow the expected postmortem rise in potassium levels, thereby extending the calculated DI beyond the actual time of death [11,12,14,16]. The consistency of potassium as a reliable marker, even under varying conditions, underscores its value in forensic investigations, although the impact of the storage conditions must be carefully considered.

On the other hand, the study observed that other biochemical markers, such as sodium and chloride in both vitreous humor and synovial fluid, showed a negative correlation with PMI, leading to underestimation of DI. The underestimations ranged from 31 to 5 min, which could be attributed to the reduced postmortem diffusion and redistribution of these electrolytes under cold-storage conditions. These findings suggest that, while sodium and chloride can provide supplementary information, their utility as primary indicators of PMI may be limited under certain conditions, such as cold storage [15,29-34]. The variability in these estimates indicated that environmental factors significantly influenced the reliability of these markers.

Interestingly, glucose levels in both fluids did not show a statistically significant correlation with PMI, with overestimations and underestimations being minimal and statistically insignificant, respectively.

This suggests that glucose may be less reliable as a PMI marker, particularly for bodies stored under cold conditions. The lack of a significant change in glucose levels could be due to its rapid postmortem consumption by residual cellular activity or microbial action, which may be less affected by cold storage [10,16]. This finding further supports the limited role of glucose in PMI estimation, especially in cases of delayed discovery and cold storage.

In summary, this study highlighted the complexities of using biochemical markers for PMI estimation in bodies stored under cold conditions. The positive correlation between potassium and PMI reaffirms its value, but the potential for overestimation must be accounted for in forensic analyses. Meanwhile, the variability and general underestimation

associated with sodium and chloride, and the non-significant role of glucose suggest that a multi-marker approach may be necessary for more accurate PMI estimations in such cases. Future research should focus on refining regression models to better account for the effects of cold storage and exploring additional markers that could improve the accuracy of PMI estimations under varying environmental conditions.

Conclusion

This study underscores the importance of considering cold storage conditions when estimating PMI using biochemical markers in vitreous humor and synovial fluid. While potassium remains a valuable marker, its tendency to overestimate DI during cold storage necessitates careful interpretation. Sodium and chloride, although informative, tend to underestimate PMI, and their use as standalone indicators may be limited. The non-significant role of glucose further complicates PMI estimation in cold-stored bodies. Therefore, death investigators should apply a multi-marker approach that considers specific environmental conditions to enhance the accuracy of DI estimates. Further research is needed to develop more robust models to mitigate the effects of cold storage on biochemical markers.

Conflicts of Interest

Declared none

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