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Original Research Article

A Study on Estimation of Time since Death by Post Mortem Examination of Electrolyte Changes in Vitrous Humor

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Abstract:

Introduction: Estimating PMI is crucial in forensic science for solving cases and identifying bodies. Electrolyte changes in the vitreous humor, including K+, Na+, and Ca+, offer potential markers for PMI determination due to predictable postmortem alterations. This study delves into the estimation of time since death by investigating the electrolyte changes in the vitreous humor, aiming to contribute to the advancement of forensic science and the accuracy of PMI determination.

Material and Method: This cross-sectional study was conducted between April 2021 and March 2022, with 100 subjects. Cases with specific conditions were excluded. Vitreous humor samples were collected and analyzed for electrolytes using an automated analyzer. Time since death (TSD) was estimated using the Sturner and Gantner formula, and correlations between PMIs were analyzed using SPSS software. The study was carried out at NAMO Medical Education & Research Institute and Shri Vinoba Bhave Civil Hospital, U.T. of Dadra & Nagar Haveli and Daman & Diu. The time of death data was obtained from police files, hospital records, or eyewitness accounts and cross-referenced with postmortem alterations for validation.

Results: In the results, we found moderate and statistically significant correlations between potassium (K+) levels and eye locations, with R values approximately 0.455 for Rt eye, 0.436 for Lt eye, and 0.554 for Both eye. For calcium (Ca+), the correlations were moderate and significant, with R values around 0.280 for Rt eye, 0.298 for Lt eye, and 0.523 for Both eye. However, correlations for sodium (Na+) levels were relatively low and not statistically significant, with R values of approximately 0.026 for Rt eye, 0.024 for Lt eye, and 0.031 for Both eye.

Conclusion: In conclusion, potassium and calcium levels showed significant correlations with eye locations, suggesting their potential as markers for postmortem interval estimation. Sodium levels did not exhibit strong associations. Comprehensive approaches are vital for accurate time of death determination.

Keywords: Postmortem interval, Time since death, Electrolyte changes.

Introduction

The estimation of time since death (TSD) is a crucial aspect in forensic medicine and forensic pathology, as it aids in solving criminal cases, identifying unknown bodies, and providing closure to families of the deceased.[1] Forensic scientists and pathologists have long sought reliable and accurate methods to determine the time elapsed since death, as conventional techniques like rigor mortis and body temperature measurements have proven to be less precise in certain circumstances.[2] One promising avenue of research in this field is the analysis of electrolyte changes in the vitreous humor, the clear gel-like substance found in the eye's posterior chamber, which has shown potential as a valuable indicator of TSD.[3] Electrolytes, such as sodium, potassium, chloride, and calcium, play vital roles in maintaining cellular function and homeostasis.[4] After death, the cellular membranes lose their integrity, leading to the release of intracellular electrolytes into the surrounding tissues and fluids. Consequently, the vitreous humor, which is isolated from the external environment, acts as a reservoir for these ions. The postmortem changes in the vitreous electrolyte concentrations have been hypothesized to exhibit a predictable pattern over time, providing forensic experts with valuable information to estimate the time of death with greater accuracy.[5]

Furthermore, the utilization of electrolyte changes in the vitreous humor as a marker for TSD estimation offers distinct advantages over traditional methods. Unlike external signs of decomposition, such as rigor mortis, which are influenced by external factors and may be affected by the cause of death, vitreous electrolyte analysis remains relatively unaffected by such variables.[6] Moreover, the vitreous humor is relatively well-protected within the eye, making it less susceptible to postmortem contamination or degradation. As a result, this method may prove to be more reliable and applicable across a broader range of forensic cases, including those involving decomposed or partially skeletonized remains, where conventional techniques often fail to provide accurate estimates.[7] The potential combination of vitreous electrolyte analysis with other established forensic tools could revolutionize TSD estimation and significantly improve the overall accuracy of forensic investigations.

Several studies have already explored the potential relationship between electrolyte changes in vitreous humor and TSD estimation.[3,8] However, the results have been inconsistent and require further investigation and validation. Factors such as environmental conditions, body temperature at the time of death, and postmortem interval can all influence the rate and pattern of electrolyte changes in the vitreous humor.[9,10] Thus, a comprehensive and systematic study is needed to elucidate the underlying mechanisms and establish a reliable model for TSD estimation based on vitreous electrolyte analysis.

The objectives of this study are twofold: firstly, to analyze the temporal changes in vitreous humor electrolyte concentrations over a postmortem period using a well-defined and controlled experimental design, and secondly, to develop a mathematical model that correlates these changes to the time elapsed since death. To achieve these objectives, a diverse and representative sample of postmortem cases will be selected, considering various factors like age, sex, cause of death, and postmortem interval. The electrolyte levels will be analyzed at regular intervals postmortem, and statistical analyses will be employed to identify significant trends and patterns.

Material and Methods

This cross-sectional study was conducted in the mortuary under the Department of Forensic Medicine at NAMO Medical Education & Research Institute and Shri VinobaBhave Civil Hospital, U.T. of Dadra & Nagar Haveli and Daman & Diu, between April 2021 and March 2022. The study was conducted following the guidelines of the Institutional Ethics Committee of NAMO Medical Education & Research Institute and Shri VinobaBhave Civil Hospital.

A total of 100 subjects were included in the study. The cases selected for analysis were those involving sudden or unnatural death, excluding individuals with a previous history of eye or orbital injury, ocular surgeries, posterior segment diseases, charred/skeletonized bodies, extreme changes of decompositions, and pediatric cases (less than 12 years of age). All subjects met the inclusion criteria and underwent postmortem examination at the designated mortuary affiliated with the Department of Forensic Medicine.

Vitreous humor samples (2-3ml) were collected from the eye using a 21-gauge needle in a 10ml syringe from the outer canthus. To prevent dislodged retinal tissue and contamination, care was taken during sample collection. Turbid blood-filled samples were discarded to ensure accuracy. After aspiration, the empty vitreous cavity was refilled with normal saline to maintain the shape of the eyeball. The obtained vitreous humor samples were immediately sent to the Central Laboratory, Department of Biochemistry, at NAMO Medical Education & Research Institute and Shri VinobaBhave Civil Hospital for analysis of electrolytes. An automated electrolyte analyzer (Accu-Life, Compact Diagnostics India Pvt. Ltd., New Delhi, India) was employed for this purpose.

In addition to vitreous humor analysis, data on the time of death were collected from police files, hospital records, or eyewitnesses, kin, friends, and other close associates of the deceased. The time since death (TSD) recorded on police records was cross-referenced with postmortem alterations, such as rigor mortis, to validate the estimations. TSD was estimated using the formula proposed by Sturner and Gantner (1964) for each electrolyte. The correlation between PMIs calculated from different electrolyte values, PMIs estimated using physical signs, and PMIs determined using official police records was analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 20 (IBM Corp., Armonk, NY).

Results

The study comprised 100 deceased individuals, and their vitreous humor samples were analyzed to assess electrolyte levels. Table1 presents the baseline characteristics of the study population. The participants' ages ranged from 13 to over 75 years, with the largest groups being in the 46-55 and 36-45 age ranges. The study population consisted of 59% males and 41% females. The main causes of death were asphyxia (38%), accidental trauma (22%), and poisoning (11%), while 29% of the cases had an unknown cause of death.

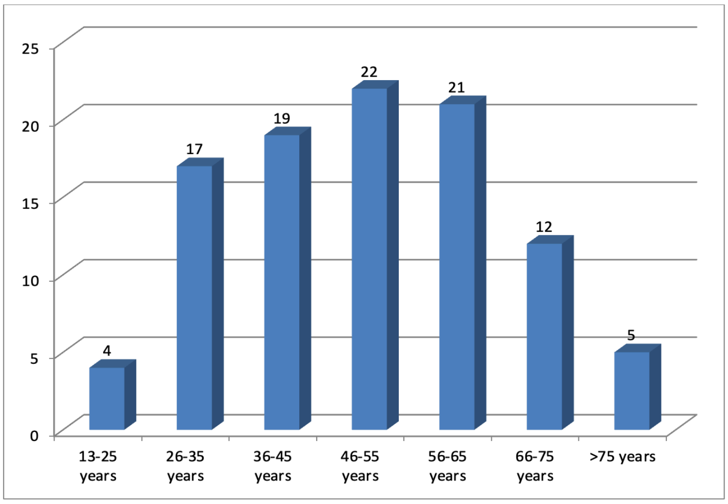

Figure 1: Different age groups in study population (%)

The table 1 represents the levels of sodium (Na+), potassium (K+), and calcium (Ca+) in the vitreous humor samples from individual eyes (right and left) and both eyes combined. The data shows that sodium levels are similar between the eyes, with mean values ranging from approximately 142.54 to 143.88 and moderate variability (standard deviation around 19.95 to 21.78). For potassium, there is slight variability in mean values, ranging from 13.86 to 14.51, with a moderate spread (standard deviation around 6.70 to 7.19). Calcium levels also exhibit minor differences between eyes, with mean values around 6.55 to 7.16 and moderate variability (standard deviation around 1.45 to 1.78). The combined analysis of both eyes (Both eye) generally aligns with the individual eye results.

Table 1: Descriptive Statistic constitute eye

Constituent	Location	N	Mean ± SD	Min	Max
Na+	Rt eye	100	142.73 ± 19.95	101	211
Na+	Lt eye	100	143.88 ± 21.78	115	274
Na+	Both eye	200	142.54 ± 20.97	108	272
K+	Rt eye	100	14.51 ± 7.19	4.7	34
K+	Lt eye	100	13.95 ± 6.70	4.8	33
K+	Both eye	200	13.86 ± 6.93	4.8	32
Ca+	Rt eye	100	6.55 ± 1.45	3.7	12.3
Ca+	Lt eye	100	7.16 ± 1.78	3.8	12.4
Ca+	Both eye	200	6.85 ± 1.67	3.7	11.5

In this study, correlations between Sodium (Na+), Potassium (K+), and Calcium (Ca+) levels in different eye locations (Rt eye, Lt eye, and Both eye) were examined. (Table 2) The correlations for Na+ were relatively low and not statistically significant, with R values approximately 0.026 for Rt eye (P > 0.05), 0.024 for Lt eye (P > 0.05), and 0.031 for Both eye (P > 0.05).

Table 2: Correlation and Significance of Electrolyte Levels with PMI in Eye Locations

Constituent	Location	N	R	SEE	P value
Na+	Rt eye	100	0.026	36.35	0.425
Na+	Lt eye	100	0.024	36.26	0.482
Na+	Both eye	200	0.031	36.14	0.549
K+	Rt eye	100	0.455	28.54	<0.001
K+	Lt eye	100	0.436	27.06	<0.001
K+	Both eye	200	0.554	28.67	<.0001
Ca+	Rt eye	100	0.280	37.75	<0.01
Ca+	Lt eye	100	0.298	37.88	<0.01
Ca+	Both eye	200	0.523	35.88	<0.01

On the other hand, K+ showed moderate to strong correlations, which were statistically significant, with R values approximately 0.455 for Rt eye ($P < 0.001$), 0.436 for Lt eye ($P < 0.001$), and 0.554 for Both eye ($P < 0.0001$). The regression equations for K+ were $y = 0.104x + 10.69$, and the coefficient of determination (R^2) was 0.307, indicating that around 30.7% of the variation in K+ levels could be explained by the eye location.

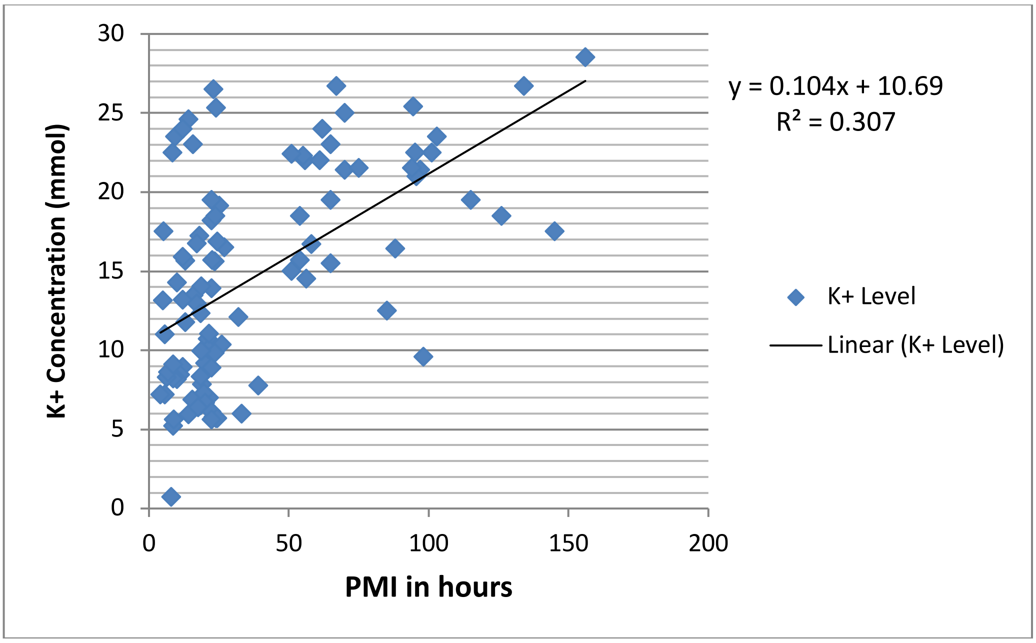

Figure 2: Linear regression correlation of vitreous K^+ and PMI

For Calcium, the correlations were moderate and statistically significant, with R values around 0.280 for Rt eye ($P < 0.01$), 0.298 for Lt eye ($P < 0.01$), and 0.523 for Both eye ($P < 0.01$). The regression equation for Calcium was $y = 0.017x + 6.148$, and the coefficient of determination (R^2) was 0.274, suggesting that approximately 27.4% of the variation in Calcium levels could be explained by the eye location. The standard error of estimate (SEE) was used to assess the accuracy of predictions. K+ exhibited relatively accurate predictions, with SEE values around 28.54 for Rt eye, 27.06 for Lt eye, and 28.67 for Both eye.

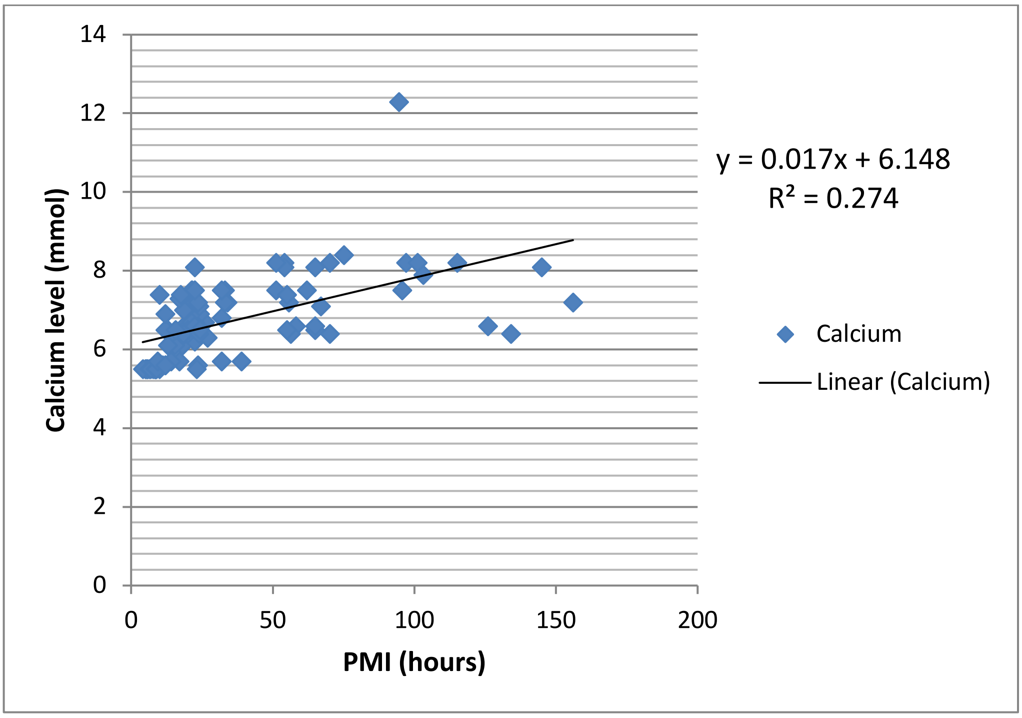

Figure 3: Linear regression correlation of vitreous calcium and PMI

Discussion

The estimation of the postmortem interval (PMI) is a crucial aspect in forensic science, aiding in various investigations. Electrolyte concentrations in the vitreous humor have been explored as potential markers for estimating the time since death, as they can undergo alterations in a predictable manner during the postmortem interval.

The study investigated the estimation of time since death by examining electrolyte changes in the vitreous humor, with a particular focus on potassium (K+) levels. The mean potassium values exhibited slight variability, ranging from 13.86 to 14.51, with a moderate spread indicated by standard deviations around 6.70 to 7.19. These differences in mean values could be attributed to various factors, such as age, health status, or diet, impacting potassium levels among individuals.

To gain further insights, correlations between potassium (K+), sodium (Na+), and calcium (Ca+) levels were examined across different eye locations (Right eye, Left eye, and Both eyes). The results revealed moderate to strong correlations, which were statistically significant. The regression equations for potassium provided valuable information, indicating that approximately 30.7% of the variation in potassium levels could be explained by the eye location. Such correlations between potassium levels and eye locations may indicate variations in potassium distribution within the vitreous humor of different eyes.

The study's findings were compared to those of other relevant research. For instance, Chand et al.[11] reported an average vitreous potassium concentration of 13.86 mmol/L, aligning closely with the present study's mean values. They also found a highly significant correlation between vitreous potassium and the postmortem interval (PMI), which is consistent with the present study's observations.

Furthermore, Pounder et al.[12] discovered significant differences in vitreous potassium levels between the two eyes of the same individual, potentially supporting the present study's findings of varying potassium levels in different eye locations. Additionally, Kumar et al.[3] noted a linear rise in vitreous potassium with increasing PMI, which concurs with the present study's outcomes. They reported a significant linear correlation between vitreous potassium and PMI, reinforcing the usefulness of potassium levels as an indicator for estimating the time since death. Kurup et al.[13] highlighted the reliability of potassium measurements in the vitreous humor for estimating PMI. These measurements offer improved accuracy and precision and remain unaffected by external factors. The linear increase in vitreous potassium with the rise of PMI in various studies[14,15], including the present one, has been attributed to the autolysis of the vascular choroids and retinal cells of the eye, resulting in the release of potassium into the vitreous humor.[16]

In our study, we found moderate and statistically significant correlations between calcium levels and eye locations. The regression equation allowed us to predict calcium concentration based on the eye location, with approximately 27.4% of the variation in calcium levels explained by the eye location. Similarly, Chand et al.[11]'s study demonstrated a significant linear regression correlation between vitreous calcium levels and postmortem interval (PMI), supporting the potential relationship between calcium concentrations and time since death.

However, Siddamsetty et al.[3]'s study did not find any significant relationship between calcium levels and time since death (TSD), contrasting with our findings and those of Chand et al.[11] Moreover, the comparison with other studies, such as Madea et al.[17] and Coe et al.[18], revealed discrepancies, with these studies reporting no relationship between calcium concentration in the vitreous humor and time since death. The varying results underscore the complexity of estimating the postmortem interval solely based on calcium levels, influenced by individual variability, body condition after death, and potential interactions with other electrolytes.

In our study, we examined the correlations for sodium (Na+) levels in the vitreous humor and their relationship with eye locations. The correlations were relatively low and not statistically significant, with R values of approximately 0.026 for Rt eye, 0.024 for Lt eye, and 0.031 for Both eye. This suggests that there is no strong association between sodium levels and eye locations.

In Chand et al.[11]'s study, the correlation between vitreous sodium and postmortem interval (PMI) was not found to be significant. The study reported a range of vitreous sodium concentrations from 98 mmol/L to 284 mmol/L, with an average of 143.10 mmol/L. Siddamsetty et al.[3]'s study revealed a weak negative relationship between time since death (TSD) and sodium levels in the vitreous humor, with a correlation coefficient of -0.137 and a p-value of 0.048. This indicates that sodium levels may slightly decrease over time after death. Balasooriya et al.[19]'s study did not find any correlation between sodium concentration in the vitreous humor and time since death. These results suggest that sodium may not be a strong indicator for estimating the postmortem interval, and other factors may play a more significant role in this estimation.

Some limitations of our study include a relatively small sample size, which may limit the generalizability of the findings. Additionally, the study focused on potassium, sodium, and calcium levels, but other electrolytes or factors that could influence PMI were not considered. The examination of eye locations might not fully capture variations in electrolyte distribution within the vitreous humor. Moreover, the study was limited to in vitro analyses, and the influence of external factors on electrolyte changes in a real-life forensic setting was not assessed.

Conclusion

In conclusion, our study focused on the estimation of time since death by examining electrolyte changes in the vitreous humor, particularly potassium, sodium, and calcium levels. We found moderate and statistically significant correlations between potassium levels and eye locations, indicating its potential as a marker for postmortem interval estimation. While calcium showed a relationship with PMI, sodium did not exhibit significant correlations, highlighting the need for caution in using it as a reliable marker for time of death estimation. Considering multiple factors and adopting a comprehensive approach is crucial for accurate postmortem interval determination.

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