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ESTIMATION OF TIME SINCE DEATH (POST MORTEM INTERVAL) BY ANALYSING POTASSIUM LEVELS IN VITREOUS HUMOR

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ABSTRACT **Aims And Objectives:** To estimate the time since death by analysing potassium (K^+) level changes in vitreous humor samples. **Methodology:** A cross-sectional study was conducted in the Department of Forensic Medicine, Osmania General Hospital and College, Hyderabad, between July and August 2022. Deceased individuals meeting inclusion and exclusion criteria were included. Vitreous humor samples were collected from one eye, and potassium levels were determined using an autoanalyzer. Post-mortem intervals (PMIs) were computed using potassium values and compared with PMIs estimated from physical signs and official police records. Data entry was done in MS Excel and analysed using SPSS software (version 20). **Results:** Of the 100 deceased individuals included, 66% were men, with the majority (25%) aged 53–62 years. A linear relationship existed between vitreous potassium concentration and PMI. No correlation was observed between vitreous potassium levels and ambient temperature. Potassium-based PMI showed high agreement with police records and physical signs (Spearman's rho statistically significant at $p < 0.01$; kappa = 0.832). **Conclusion:** Potassium concentration in vitreous humour provides a precise and reliable estimate of PMI, unaffected by external factors, and can be used as a dependable tool in forensic practice.

KEYWORDS : post-mortem interval (PMI), vitreous humour, potassium, time since death.

INTRODUCTION

An autopsy is a useful tool to establish an accurate diagnosis when a person's death is attributed to unknown causes [1]. Estimating the time since death (TSD)/Post-Mortem Interval (PMI) during an autopsy becomes crucial because it helps determine the proper civil and criminal penalties. It is just the computation of a date and time interval that can be measured and computed along a time-dependent curve that is expressed within an interval range [2]. Autopsy surgeons frequently rely on the highly subjective physical indicators of death. As a result, the PMI's accuracy and dependability are in danger[3]. Numerous internal and external factors, including the existence of an underlying illness, the surrounding environment's influence on temperature and humidity, and the last terminal action's thermodynamics, can affect TSD. In these situations, more modern techniques like chemical analysis help determine TSD[4-7]. Numerous biochemical alterations begin to take place during the agonising phases of death. These are commonly attributed to lowered blood oxygen levels, altered enzyme reactions, and a halt in the metabolite production process. Similar fluctuations are seen in all physiological fluids, and measurements of these metabolite profiles can help determine how much time has passed[8-11]. Vitreous Humour is one such typical bodily fluid. Commonly studied electrolytes for estimating TSD include potassium, hypoxanthine, and lactic acid; these studies are primarily focused on cases of natural death.[12-15]. After death, the vascular choroidal and retinal cells of the eye undergo autolysis, which is thought to be the cause of the gradual rise in potassium concentration in the vitreous[13,14]. In the current study, we sought to examine the potassium electrolyte changes that occur in the vitreous humour and examine their impact on estimating the PMI in cases of unnatural death, given the Humor's accessibility and resistance to putrefaction.

MATERIALS AND METHODS

A cross-sectional study was conducted in the Department of Forensic Medicine, Osmania General Hospital and College, Hyderabad, between July and August 2022. After obtaining Institutional Ethics Committee (IEC) approval (IEC/OMC/MEETINGNUMBER11/ACADEMIC-121), the subjects were recruited. The ethical committee allowed an exemption for obtaining informed consent, so no separate written consent was obtained from the patient party.. After all, any procedure about postmortem would include proper estimation of TSD, and any such investigation is a part of routine postmortem examination. The study included all case reported as death or unnatural death (death not attributable to any underlying disease) in which a postmortem was conducted in the mortuary connected to the Department of Forensic Medicine of the approved setting. Cases with a prior history of orbital or eye injuries, ocular surgeries, diseases of the posterior segment, cases of unnatural death with charred or skeletonized bodies, bodies retrieved in extreme states of decomposition, and paediatric cases (less than 12 years old), given the small amount of vitreous fluid in them, were all excluded.

No particular sampling technique was used for the study because of its brief duration and the lack of sufficient literature on the subject in the geographic region that was described (geography influences ambient temperatures and the vitreous potassium values indirectly). The study included every deceased person who satisfied the inclusion requirements and did not fit under the exclusion criteria. Samples of vitreous humour were gradually and carefully aspirated from the posterior chamber through a puncture made 5–6 mm away from the limbus (ora serrata) using a sterile 20-gauge needle[12,13]. Samples containing turbid blood were discarded. The study's principal investigator underwent preparatory simulation training to finish the technique on cadaveric specimens; however, those samples were not used in the investigation. The collected vitreous humour samples were sent right away to our hospital's Central Laboratory, Department of Biochemistry so that an automated electrolyte analyzer could measure the vitreous potassium levels[14,15]. Additionally, information regarding the time of death was obtained from police files, hospital records, eyewitnesses, kin, friends, and other close associates of the deceased. The TSD determined in this manner was then double-checked using postmortem alterations such as rigor mortis. The formula proposed by Sturner and Gantner (1964) was applied as follows: PMI (hrs) = 7.14 K+ (mEq/L) - 39.1. PMI stands for PMI, and K+ stands for vitreous tumor potassium concentration[18]. The correlation between PMIs estimated from physical signs, PMIs calculated from potassium values, and PMIs determined from official police records was established using Spearman's rho. The Statistical Package for the Social Sciences (SPSS) software, version 20 (IBM Corp., Armonk, NY), was used to analyse the data after they were entered into Ms Excel 2020 (Microsoft Corporation, Redmond, Washington). Categorical variables are expressed as the mean ± standard deviation and percentages, while comparative data are expressed as ratios and proportions.

Inclusion Criteria:

1. Deaths due to unnatural causes undergoing autopsy.

Exclusion Criteria:

1. Orbital/ocular injury cases
2. Ocular surgeries or posterior segment pathology

3. Charred, skeletonised or decomposed bodies
4. Children <12 years (insufficient vitreous volume).

Vitreous humour was aspirated using a sterile 20-gauge needle from the posterior chamber, avoiding blood contamination. Samples were analysed using an automated electrolyte analyser. Time of death was cross-verified with police reports, hospital records, and physical signs (rigor mortis).

Formula used (Sturner & Gantner, 1964):

$$PMI\ (hours) = 7.14 \times K^{+}(mEq/L) - 39.1$$

Spearman's rho correlation was applied between PMI estimates. Data analysis was performed using SPSS v20.

RESULTS

The study comprised one hundred deceased subjects whose vitreous humor samples were examined for potassium levels The study cohort's baseline characteristics are Listed in Table 1.

Table 1 : Age Characteristics Of Study Population N =100

Age groups	Frequency
13-23 years	5
23-32 years	14
33-42 years	17
43-52 years	22
53-62 years	25
63-72 years	15
73-82 years	2
>83 years	0

The study population was primarily composed of men (66%) and deceased individuals in the age range of 53–62 years (25%) represented in Table 1.

Unclassified deaths account for the majority of deaths (40%), asphyxia (22%)followed by trauma (38%). Table 3 presents the basic distribution of the PMI

Based on potassium levels and physical signs (rigor mortis) from official police Records.

Table 2: Police official records, physical signs (rigor mortis), and potassium values in the vitreous humour were used to determine the postmortem interval (PMI).

Parameters	PMI using potassium values	PMI using police official records	PMI using rigor mortis	Ambient temperature
Mean ± standard deviation	14.2 ± 8.56	14.1 ± 8.29	14.0 ± 8.43	30.5 ± 2.47
Median	13.2	14.6	13.5	32
Interquartile range	(7.1, 21.4)	(5.8, 21)	(6.3, 19.2)	(30.1, 34)

Additionally, it shows information about the current ambient temperature, which may affect how TSD is determined. Moreover, no relationship was found between the vitreous humor's potassium levels and the ambient temperature that is, the temperature from which the body is recovered.

Table 3: Showing Correlations Between Different Variables.

			PMI from police official records	PMI by potassium values in vitreous humor	PMI by physical signs (rigor mortis)
Spearma n's rho	PMI from police official records	Correlation Coefficient	1.000	0.940**	0.900**
		Sig. (2-tailed)		0.000	0.000
		N	100	100	100
	PMI by potassium values in vitreous humor	Correlation Coefficient	0.940**	1.000	0.812**
		Sig. (2-tailed)	0.000		0.000
		N	100	100	100
	PMI by physical signs (rigor mortis)	Correlation Coefficient	0.900**	0.812**	1.000
		Sig. (2-tailed)	0.000	0.000	
		N	100	100	100

The two-tailed significance level of the **correlation is 0.01. Postmortem interval (PMI).The PMI determined by each method shows little variation, with all three findings being statistically significant at the 0.01 or two-tailed level. As a result, the PMI provided by the physical indicators and police records is distinct from the PMI verified by the potassium levels. The agreement between the PMIs estimated from the Police records and the potassium values is also shown in Table 4. The recorded PMI and potassium levels are almost perfectly in agreement, as indicated by the kappa value of 0.832. Given the previously mentioned results, we can affirm that estimating the TSD/PMI by measuring potassium levels is still a highly effective method because there isn't much agreement between PMI estimated by police official records and PMI derived from potassium values in the vitreous humor.

DISCUSSION

Every criminal investigation needs to use TSD estimation. After death, the body changes in an orderly manner due to physiochemical, biochemical, autolytic, metabolic, and physical processes. These changes can be utilised to calculate TSD[16]. Over the past few years, several formulas have been developed to calculate this interval by combining different statistical techniques with the concentrations of compounds found in vitreous humour[17]. In this study, we investigated the viability of using vitreous potassium levels as a marker of PMI in deceased individuals. Variables like the surrounding temperature, which can alter the concentration of the substances present in the vitreous humour, were taken into account when estimating the TSD. The results of this investigation demonstrated a positive linear correlation between vitreous potassium concentration and PMI. With values of 0.900, 0.940, and 0.812 at the 0.01 or two-tailed level, Spearman's rho was statistically significant, indicating that the potassium levels in the vitreous humour increased as the TSD increased. The findings of W.W. Jetter et al, W.G. Robertson et al., R. Nowak et al, and Madea et al. are corroborated by these results[19-23].

The majority of these studies suggested models for linear regression. R. Nowak et al and Jashnani et al also found an inverse correlation between the vitreous sodium/potassium ratio and the PMI. While Cordeiro et al. proposed using vitreous hypoxanthine and urea levels to calculate the amount of TSD, James et al. found that these levels can also be used to estimate the PMI. His research also emphasised the application of PMI-generating software from these electrolytes[21-23].

The present study found no significant correlation between the vitreous humour potassium values and age, sex, ambient temperature, or cause of death. Furthermore, there was a higher correlation (kappa coefficient of 0.88) between the TSD determined by Sturner's proposed formula and the TSD determined by police records, the physical signs of Rigor mortis, and other factors. Our study's findings support those of Jashnani et al. along with W.W. Jetter et al, who assert that variables like sample age and sex had no effect on the potassium levels in the vitreous humour[19,23]. This study found no correlation between temperature and potassium concentration levels in the vitreous humour following death, raising questions about the role of temperature in these levels. This result is consistent with findings by W.W. Jetter et al, W.G. Robertson et al., R. Nowak et al, and Madea et al and others who discovered that seasonal variation did not affect the increase in potassium levels after death[17,19,20.21,22,24].

Limitations Of Study

To demonstrate the accuracy of fluid electrolyte changes after death in determining PMI, this study was limited to cases of deaths and included a comparison of PMI determined by vitreous potassium with official police records and physical signs of death. Additionally, this study adds to the scant body of research on the geographic population of South India. One of the main shortcomings of the research is the small sample size. The study's scope was constrained by the brief duration that TSD allows. In order to ascertain the value and

correlation of vitreous potassium levels in identifying PMI with diverse modes and causes of death in the future, a similar study involving a larger population and all cases of death is also planned.

CONCLUSION

There is a linear correlation between vitreous potassium concentration and TSD. The amount of potassium in the vitreous has no appreciable effect on age, gender, cause of death, or ambient temperature. Furthermore, there exists a robust correlation between The true TSD ascertained by Rigor mortis and official police records, and the estimated TSD ascertained by potassium levels in the vitreous humour. Potassium measurements are a reliable marker for PMI because they are associated with improved accuracy across all scenarios.

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